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<p>(21) International Application Number: <b>PCT/US99/14484</b></p> <p>(22) International Filing Date: <b>25 June 1999 (25.06.99)</b></p> <p>(30) Priority Data:</p> <table style="width: 100%; border-collapse: collapse;"> <tr> <td style="width: 15%;">60/090,762</td> <td style="width: 15%;">26 June 1998 (26.06.98)</td> <td style="width: 15%;">US</td> <td style="width: 15%;"></td> </tr> <tr> <td>60/094,983</td> <td>31 July 1998 (31.07.98)</td> <td>US</td> <td></td> </tr> <tr> <td>60/102,686</td> <td>1 October 1998 (01.10.98)</td> <td>US</td> <td></td> </tr> <tr> <td>60/112,129</td> <td>11 December 1998 (11.12.98)</td> <td>US</td> <td></td> </tr> </table> <p>(63) Related by Continuation (CON) or Continuation-in-Part (CIP) to Earlier Applications</p> <table style="width: 100%; border-collapse: collapse;"> <tr> <td style="width: 15%;">US</td> <td style="width: 15%;">60/090,762-(CIP)</td> <td style="width: 15%;"></td> </tr> <tr> <td>Filed on</td> <td>26 June 1998 (26.06.98)</td> <td></td> </tr> <tr> <td>US</td> <td>60/094,983 (CIP)</td> <td></td> </tr> <tr> <td>Filed on</td> <td>31 July 1998 (31.07.98)</td> <td></td> </tr> <tr> <td>US</td> <td>60/102,686 (CIP)</td> <td></td> </tr> <tr> <td>Filed on</td> <td>1 October 1998 (01.10.98)</td> <td></td> </tr> <tr> <td>US</td> <td>60/112,129 (CIP)</td> <td></td> </tr> <tr> <td>Filed on</td> <td>11 December 1998 (11.12.98)</td> <td></td> </tr> </table> <p>(71) Applicant (for all designated States except US): <b>INCYTE PHARMACEUTICALS, INC. [US/US]; 3174 Porter Drive, Palo Alto, CA 94304 (US).</b></p>				60/090,762	26 June 1998 (26.06.98)	US		60/094,983	31 July 1998 (31.07.98)	US		60/102,686	1 October 1998 (01.10.98)	US		60/112,129	11 December 1998 (11.12.98)	US		US	60/090,762-(CIP)		Filed on	26 June 1998 (26.06.98)		US	60/094,983 (CIP)		Filed on	31 July 1998 (31.07.98)		US	60/102,686 (CIP)		Filed on	1 October 1998 (01.10.98)		US	60/112,129 (CIP)		Filed on	11 December 1998 (11.12.98)	
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<p>(54) Title: <b>HUMAN SIGNAL PEPTIDE-CONTAINING PROTEINS</b></p> <p>(57) Abstract</p> <p>The invention provides human signal peptide-containing proteins (HSPP) and polynucleotides which identify and encode HSPP. The invention also provides expression vectors, host cells, antibodies, agonists, and antagonists. The invention also provides methods for diagnosing, treating, or preventing disorders associated with expression of HSPP.</p>																																											

## HUMAN SIGNAL PEPTIDE-CONTAINING PROTEINS

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## TECHNICAL FIELD

This invention relates to nucleic acid and amino acid sequences of human signal peptide-containing proteins and to the use of these sequences in the diagnosis, treatment, and prevention of cell proliferative disorders including cancer; inflammation; and cardiovascular, neurological, 10 reproductive, and developmental disorders.

## BACKGROUND OF THE INVENTION

Protein transport is essential for cellular function. Transport of a protein may be 15 mediated by a signal peptide located at the amino terminus of the protein itself. The signal peptide is comprised of about ten to twenty hydrophobic amino acids which target the nascent protein from the ribosome to a particular membrane bound compartment such as the endoplasmic reticulum (ER). Proteins targeted to the ER may either proceed through the secretory pathway or remain in any of the secretory organelles such as the ER, Golgi 20 apparatus, or lysosomes. Proteins that transit through the secretory pathway are either secreted into the extracellular space or retained in the plasma membrane. Secreted proteins are often synthesized as inactive precursors that are activated by post-translational processing events during transit through the secretory pathway. Such events include glycosylation, phosphorylation, proteolysis, and removal of the signal peptide by a signal 25 peptidase. Other events that may occur during protein transport include chaperone-dependent unfolding and folding of the nascent protein and interaction of the protein with a receptor or pore complex. Examples of secreted proteins with amino terminal signal peptides are discussed below and include receptors, extracellular matrix molecules, cytokines, hormones, growth and differentiation factors, neuropeptides, vasomediators, 30 phosphokinases, phosphatases, phospholipases, phosphodiesterases, G and Ras-related proteins, ion channels, transporters/pumps, proteases, and transcription factors. (Reviewed in Alberts, B. et al. (1994) Molecular Biology of The Cell, Garland Publishing, New York, NY, pp. 557-560, 582-592.)

G-protein coupled receptors (GPCRs) comprise a superfamily of integral membrane proteins which transduce extracellular signals. GPCRs include receptors for biogenic amines such as dopamine, epinephrine, histamine, glutamate (metabotropic effect), acetylcholine (muscarinic effect), and serotonin; for lipid mediators of inflammation such as prostaglandins, platelet activating factor, and leukotrienes; for peptide hormones such as calcitonin, C5a anaphylatoxin, follicle stimulating hormone, gonadotropin releasing hormone, neurokinin, oxytocin, and thrombin; and for sensory signal mediators such as retinal photopigments and olfactory stimulatory molecules. The structure of these highly conserved receptors consists of seven hydrophobic transmembrane regions, cysteine disulfide bridges between the second and third extracellular loops, an extracellular N-terminus, and a cytoplasmic C-terminus. The N-terminus interacts with ligands, the disulfide bridges interact with agonists and antagonists, and the large third intracellular loop interacts with G proteins to activate second messengers such as cyclic AMP, phospholipase C, inositol triphosphate, or ion channels. (Reviewed in Watson, S. and Arkinstall, S. (1994) The G-protein Linked Receptor Facts Book, Academic Press, San Diego, CA, pp. 2-6; and Bolander, F.F. (1994) Molecular Endocrinology, Academic Press, San Diego, CA, pp. 162-176.)

Other types of receptors include cell surface antigens identified on leukocytic cells of the immune system. These antigens have been identified using systematic, monoclonal antibody (mAb)-based "shot gun" techniques. These techniques have resulted in the production of hundreds of mAbs directed against unknown cell surface leukocytic antigens. These antigens have been grouped into "clusters of differentiation" based on common immunocytochemical localization patterns in various differentiated and undifferentiated leukocytic cell types. Antigens in a given cluster are presumed to identify a single cell surface protein and are assigned a "CD" number. Some of the genes encoding proteins identified by CD antigens have been isolated and characterized as both transmembrane proteins and cell surface proteins anchored to the plasma membrane via covalent attachment to fatty acid-containing glycolipids such as glycosylphosphatidylinositol (GPI). (Reviewed in Barclay, A. N. et al. (1993) The Leucocyte Antigen Facts Book, Academic Press, San Diego, CA, pp. 144-145; Noel, L. S. et al. (1998) *J. Biol. Chem.* 273:3878-3883.)

Tetraspanins are a superfamily of membrane proteins which facilitate the formation

and stability of cell-surface signaling complexes containing lineage-specific proteins, integrins, and other tetraspanins. They are involved in cell activation, proliferation (including cancer), differentiation, adhesion, and motility. These proteins cross the membrane four times, have conserved intracellular – and C-termini and an extracellular, 5 non-conserved hydrophilic domain. Tetraspanins include, e.g., platelet and endothelial cell membrane proteins, leukocyte surface proteins, tissue specific and tumorous antigens, and the retinitis pigmentosa-associated gene peripherin. (Maecker, H.T. et al. (1997) *FASEB J.* 11:428-442.)

Matrix proteins (MPs) are transmembrane and extracellular proteins which 10 function in formation, growth, remodeling, and maintenance of tissues and as important mediators and regulators of the inflammatory response. The expression and balance of MPs may be perturbed by biochemical changes that result from congenital, epigenetic, or infectious diseases. In addition, MPs affect leukocyte migration, proliferation, differentiation, and activation in the immune response. MPs are frequently characterized 15 by the presence of one or more domains which may include collagen-like domains, EGF-like domains, immunoglobulin-like domains, and fibronectin-like domains. In addition, some MPs are heavily glycosylated. MPs include extracellular proteins such as fibronectin, collagen, and galectin and cell adhesion receptors such as cell adhesion molecules (CAMs), cadherins, and integrins. (Reviewed in Ayad, S. et al. (1994) The 20 Extracellular Matrix Facts Book, Academic Press, San Diego, CA, pp. 2-16; Ruoslahti, E. (1997) *Kidney Int.* 51:1413-1417; Sjaastad, M.D. and Nelson, W.J. (1997) *BioEssays* 19:47-55.)

Lectins are proteins characterized by their ability to bind carbohydrates on cell 25 membranes by means of discrete, modular carbohydrate recognition domains, CRDs. (Kishore, U. et al. (1997) *Matrix Biol.* 15:583-592.) Certain cytokines and membrane-spanning proteins have CRDs which may enhance interactions with extracellular or intracellular ligands, with proteins in secretory pathways, or with molecules in signal transduction pathways. The lipocalin superfamily constitutes a phylogenetically 30 conserved group of more than forty proteins that function by binding to and transporting a variety of physiologically important ligands. (Tanaka, T. et al. (1997) *J. Biol. Chem.* 272:15789-15795; and van't Hof, W. et al. (1997) *J. Biol. Chem.* 272:1837-1841.) Selectins are a family of calcium ion-dependent lectins expressed on inflamed vascular

endothelium and the surface of some leukocytes. (Rossiter, H. et al. (1997) Mol. Med. Today 3:214-222.)

Protein kinases regulate many different cell proliferation, differentiation, and signaling processes by adding phosphate groups to proteins. Reversible protein phosphorylation is a key strategy for controlling protein functional activity in eukaryotic cells. The high energy phosphate which drives this activation is generally transferred from adenosine triphosphate molecules (ATP) to a particular protein by protein kinases and removed from that protein by protein phosphatases. Phosphorylation occurs in response to extracellular signals, cell cycle checkpoints, and environmental or nutritional stresses.

10 Protein kinases may be roughly divided into two groups; protein tyrosine kinases (PTKs) which phosphorylate tyrosine residues, and serine/threonine kinases (STKs) which phosphorylate serine or threonine residues. A few protein kinases have dual specificity. A majority of kinases contain a similar 250-300 amino acid catalytic domain. (Hardie, G. and Hanks, S. (1995) The Protein Kinase Facts Book, Vol I, pp. 7-47, Academic Press, 15 San Diego, CA.)

Protein phosphatases remove phosphate groups from molecules previously modified by protein kinases thus participating in cell signaling, proliferation, differentiation, contacts, and oncogenesis. Protein phosphorylation is a key strategy used to control protein functional activity in eukaryotic cells. The high energy phosphate is transferred from ATP to a protein by protein kinases and removed by protein phosphatases. There appear to be three, evolutionarily-distinct protein phosphatase gene families: protein phosphatases (PPs); protein tyrosine phosphatases (PTPs); and acid/alkaline phosphatases (APs). PPs dephosphorylate phosphoserine/threonine residues and are an important regulator of many cAMP mediated, hormone responses in cells.

20 PTPs reverse the effects of protein tyrosine kinases and therefore play a significant role in cell cycle and cell signaling processes. Although APs dephosphorylate substrates in vitro, their role in vivo is not well known. (Charbonneau, H. and Tonks, N.K. (1992) Annu. Rev. Cell Biol. 8:463-493.)

Cyclic nucleotides (cAMP and cGMP) function as intracellular second messengers to transduce a variety of extracellular signals, including hormones, light and neurotransmitters. Cyclic nucleotide phosphodiesterases (PDEs) degrade cyclic nucleotides to their corresponding monophosphates, thereby regulating the intracellular

concentrations of cyclic nucleotides and their effects on signal transduction. At least seven families of mammalian PDEs have been identified based on substrate specificity and affinity, sensitivity to cofactors and sensitivity to inhibitory drugs. (Beavo, J.A. (1995) *Physiological Reviews* 75: 725-748.)

5 Phospholipases (PLs) are enzymes that catalyze the removal of fatty acid residues from phosphoglycerides. PLs play an important role in transmembrane signal transduction and are named according to the specific ester bond in phosphoglycerides that is hydrolyzed, i.e., A<sub>1</sub>, A<sub>2</sub>, C or D. PLA<sub>2</sub> cleaves the ester bond at position 2 of the glycerol moiety of membrane phospholipids giving rise to arachidonic acid. Arachidonic acid is  
10 the common precursor to four major classes of eicosanoids, namely prostaglandins, prostacyclins, thromboxanes and leukotrienes. Eicosanoids are signaling molecules involved in the contraction of smooth muscle, platelet aggregation, and pain and inflammatory responses. (Alberts, B. et al. (1994) Molecular Biology of The Cell, Garland Publishing, Inc., New York, NY, pp. 85, 211, 239-240, 642-645.)

15 The nucleotide cyclases, i.e., adenylylate and guanylylate cyclase, catalyze the synthesis of the cyclic nucleotides, cAMP and cGMP, from ATP and GTP, respectively. They act in concert with phosphodiesterases, which degrade cAMP and cGMP, to regulate the cellular levels of these molecules and their functions. cAMP and cGMP function as intracellular second messengers to transduce a variety of extracellular signals, e.g.,  
20 hormones, and light and neurotransmitters. (Stryer, L. (1988) Biochemistry W.H. Freeman and Co., New York, pp. 975-980, 1029-1035.)

Cytokines are produced in response to cell perturbation. Some cytokines are produced as precursor forms, and some form multimers in order to become active. They are produced in groups and in patterns characteristic of the particular stimulus or disease,  
25 and the members of the group interact with one another and other molecules to produce an overall biological response. Interleukins, neurotrophins, growth factors, interferons, and chemokines are all families of cytokines which work in conjunction with cellular receptors to regulate cell proliferation and differentiation and to affect such activities as leukocyte migration and function, hematopoietic cell proliferation, temperature regulation, acute  
30 response to infections, tissue remodeling, apoptosis, and cell survival. Studies using antibodies or other drugs that modify the activity of a particular cytokine are used to elucidate the roles of individual cytokines in pathology and physiology.

Chemokines, in particular, are small chemoattractant cytokines involved in inflammation, leukocyte proliferation and migration, angiogenesis and angiostasis, regulation of hematopoiesis, HIV infectivity, and stimulation of cytokine secretion. Chemokines generally contain 70-100 amino acids and are subdivided into four 5 subfamilies based on the presence of conserved cysteine-based motifs. (Callard, R. and Gearing, A. (1994) The Cytokine Facts Book. Academic Press, New York, NY, pp. 181-190, 210-213, 223-227.)

Growth and differentiation factors are secreted proteins which function in 10 intercellular communication. Some factors require oligomerization or association with MPs for activity. Complex interactions among these factors and their receptors trigger intracellular signal transduction pathways that stimulate or inhibit cell division, cell differentiation, cell signaling, and cell motility. Most growth and differentiation factors act on cells in their local environment (paracrine signaling). There are three broad classes 15 of growth and differentiation factors. The first class includes the large polypeptide growth factors such as epidermal growth factor, fibroblast growth factor, transforming growth factor, insulin-like growth factor, and platelet-derived growth factor. The second class includes the hematopoietic growth factors such as the colony stimulating factors (CSFs). Hematopoietic growth factors stimulate the proliferation and differentiation of blood cells such as B-lymphocytes, T-lymphocytes, erythrocytes, platelets, eosinophils, basophils, 20 neutrophils, macrophages, and their stem cell precursors. The third class includes small peptide factors such as bombesin, vasopressin, oxytocin, endothelin, transferrin, angiotensin II, vasoactive intestinal peptide, and bradykinin which function as hormones to regulate cellular functions other than proliferation.

Growth and differentiation factors play critical roles in neoplastic transformation of 25 cells in vitro and in tumor progression in vivo. Inappropriate expression of growth factors by tumor cells may contribute to vascularization and metastasis of melanotic tumors. During hematopoiesis, growth factor misregulation can result in anemias, leukemias, and 30 lymphomas. Certain growth factors such as interferon are cytotoxic to tumor cells both in vivo and in vitro. Moreover, some growth factors and growth factor receptors are related both structurally and functionally to oncoproteins. In addition, growth factors affect transcriptional regulation of both proto-oncogenes and oncosuppressor genes. (Reviewed in Pimentel, E. (1994) Handbook of Growth Factors, CRC Press, Ann Arbor, MI, pp. 1-9.)

Proteolytic enzymes or proteases either activate or deactivate proteins by hydrolyzing peptide bonds. Proteases are found in the cytosol, in membrane-bound compartments, and in the extracellular space. The major families are the zinc, serine, cysteine, thiol, and carboxyl proteases.

5 Zinc proteases, e.g., carboxypeptidase A, have a zinc ion bound to the active site. These proteases recognize C-terminal residues that contain an aromatic or bulky aliphatic side chain, and hydrolyze the peptide bond adjacent to the C-terminal residues. Serine proteases have an active site serine residue and include digestive enzymes, e.g., trypsin and chymotrypsin, components of the complement and blood-clotting cascades, and

10 enzymes that control the degradation and turnover of extracellular matrix (ECM) molecules. Cysteine proteases (e.g. cathepsin) are produced by monocytes, macrophages and other immune cells, and are involved in diverse cellular processes ranging from the processing of precursor proteins to intracellular degradation. Overproduction of these enzymes can cause the tissue destruction associated with rheumatoid arthritis and asthma.

15 Thiol proteases, e.g., papain, contain an active site cysteine and are widely distributed within tissues. Carboxyl proteases, e.g., pepsin, are active only under acidic conditions (pH 2 to 3).

20 Guanosine triphosphate-binding proteins (G proteins) can be grouped into two major classes: heterotrimeric G proteins and small G proteins. Heterotrimeric G proteins interact with GPCRs that respond to hormones, growth factors, neuromodulators, or other signaling molecules. The interaction between GPCR and G protein allows the G protein to exchange GTP for guanosine diphosphate (GDP). This exchange activates the G protein, allowing it to dissociate from the receptor and interact with its cognate second messenger-generating protein, e.g., adenylate cyclase, guanylate cyclase, phospholipase C,

25 or ion channels. The hydrolysis of GTP to GDP by the G protein acts as an on-off switch, terminating the action of the G protein and preparing it to interact with another receptor molecule, thus beginning another round of signal transduction.

30 The small G proteins consist of single 21-30 kDa polypeptides. They can be classified into five subfamilies: Ras, Rho, Ran, Rab, and ADP-ribosylation factor. These proteins regulate cell growth, cell cycle control, protein secretion, and intracellular vesicle interaction. In particular, the Ras proteins are essential in transducing signals from receptor tyrosine kinases to serine/threonine kinases which control cell growth and

differentiation. Mutant Ras proteins, which bind but can not hydrolyze GTP, are permanently activated and cause continuous cell proliferation or cancer. All five subfamilies share common structural features and four conserved motifs. Most of the membrane-bound G proteins require a carboxy terminal isoprenyl group (CAAX), added 5 posttranslationally, for membrane association and biological activity. The G proteins also have a variable effector region, located between motifs I and II, which is characterized as the interaction site for guanine nucleotide exchange factors or GTPase-activating proteins.

Eukaryotic cells are bound by a membrane and subdivided into membrane-bound compartments. Membranes are impermeable to many ions and polar molecules, therefore 10 transport of these molecules is mediated by ion channels, ion pumps, transport proteins, or pumps. Symporters and antiporters regulate cytosolic pH by transporting ions and small molecules, e.g., amino acids, glucose, and drugs, across membranes; symporters transport small molecules and ions in the same direction, and antiporters, in the opposite direction. Transporter superfamilies include facilitative transporters and active ATP binding cassette 15 transporters involved in multiple-drug resistance and the targeting of antigenic peptides to MHC Class I molecules. These transporters bind to a specific ion or other molecule and undergo conformational changes in order to transfer the ion or molecule across a membrane. Transport can occur by a passive, concentration-dependent mechanism or can be linked to an energy source such as ATP hydrolysis or an ion gradient.

20 Ion channels, ion pumps, and transport proteins mediate the transport of molecules across cellular membranes. Symporters and antiporters regulate cytosolic pH by transporting ions and small molecules such as amino acids, glucose, and drugs. Symporters transport small molecules and ions unidirectionally, and antiporters, bidirectionally. Transporter superfamilies include facilitative transporters and active ATP- 25 binding cassette transporters which are involved in multiple-drug resistance and the targeting of antigenic peptides to MHC Class I molecules. These transporters bind to a specific ion or other molecule and undergo a conformational change in order to transfer the ion or molecule across the membrane. Transport can occur by a passive, concentration-dependent mechanism or can be linked to an energy source such as ATP 30 hydrolysis. (Reviewed in Alberts, B. et al. (1994) Molecular Biology of The Cell, Garland Publishing, New York, NY, pp. 523-546.)

Ion channels are formed by transmembrane proteins which create a lined passageway across the membrane through which water and ions, such as  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Ca}^{2+}$ , and  $\text{Cl}^-$ , enter and exit the cell. For example, chloride channels are involved in the regulation of the membrane electric potential as well as absorption and secretion of ions across the membrane. Chloride channels also regulate the internal pH of membrane-bound organelles.

Ion pumps are ATPases which actively maintain membrane gradients. Ion pumps are classified as P, V, or F according to their structure and function. All have one or more binding sites for ATP in their cytosolic domains. The P-class ion pumps include  $\text{Ca}^{2+}$  ATPase and  $\text{Na}^+/\text{K}^+$  ATPase and function in transporting  $\text{H}^+$ ,  $\text{Na}^+$ ,  $\text{K}^+$ , and  $\text{Ca}^{2+}$  ions. P-class pumps consist of two  $\alpha$  and two  $\beta$  transmembrane subunits. The V- and F-class ion pumps have similar structures and but transport only  $\text{H}^+$ . F class  $\text{H}^+$  pumps mediate transport across the membranes of mitochondria and chloroplasts, while V-class  $\text{H}^+$  pumps regulate acidity inside lysosomes, endosomes, and plant vacuoles.

A family of structurally related intrinsic membrane proteins known as facilitative glucose transporters catalyze the movement of glucose and other selected sugars across the plasma membrane. The proteins in this family contain a highly conserved, large transmembrane domain comprised of 12  $\alpha$ -helices, and several weakly conserved, cytoplasmic and exoplasmic domains (Pessin, J. E., and Bell, G.I. (1992) *Annu. Rev. Physiol.* 54:911-930).

Amino acid transport is mediated by  $\text{Na}^+$  dependent amino acid transporters. These transporters are involved in gastrointestinal and renal uptake of dietary and cellular amino acids and in neuronal reuptake of neurotransmitters. Transport of cationic amino acids is mediated by the system y<sup>+</sup> family and the cationic amino acid transporter (CAT) family. Members of the CAT family share a high degree of sequence homology, and each contains 12-14 putative transmembrane domains (Ito, K. and Groudine, M. (1997) *J. Biol. Chem.* 272:26780-26786).

Proton-coupled, 12 membrane-spanning domain transporters such as PEPT 1 and PEPT 2 are responsible for gastrointestinal absorption and for renal reabsorption of peptides using an electrochemical  $\text{H}^+$  gradient as the driving force. A heterodimeric peptide transporter, consisting of TAP 1 and TAP 2, is associated with antigen processing. Peptide antigens are transported across the membrane of the endoplasmic reticulum so

they can be presented to the major histocompatibility complex class I molecules. Each TAP protein consists of multiple hydrophobic membrane spanning segments and a highly conserved ATP-binding cassette. (Boll, M. et al. (1996) Proc. Natl. Acad. Sci. 93:284-289.)

5       Hormones are secreted molecules that travel through the circulation and bind to specific receptors on the surface of, or within, target cells. Although they have diverse biochemical compositions and mechanisms of action, hormones can be grouped into two categories. One category consists of small lipophilic hormones that diffuse through the plasma membrane of target cells, bind to cytosolic or nuclear receptors, and form a 10 complex that alters gene expression. Examples of these molecules include retinoic acid, thyroxine, and the cholesterol-derived steroid hormones such as progesterone, estrogen, testosterone, cortisol, and aldosterone. The second category consists of hydrophilic hormones that function by binding to cell surface receptors that transduce signals across the plasma membrane. Examples of such hormones include amino acid derivatives such 15 as catecholamines and peptide hormones such as glucagon, insulin, gastrin, secretin, cholecystokinin, adrenocorticotrophic hormone, follicle stimulating hormone, luteinizing hormone, thyroid stimulating hormone, and vasopressin. (See, for example, Lodish et al. (1995) Molecular Cell Biology, Scientific American Books Inc., New York, NY, pp. 856-864.)

20       Neuropeptides and vasomediators (NP/VM) comprise a large family of endogenous signaling molecules. Included in this family are neuropeptides and neuropeptide hormones such as bombesin, neuropeptide Y, neuropeptid N, neuromedin N, melanocortins, opioids, galanin, somatostatin, tachykinins, urotensin II and related peptides involved in smooth muscle stimulation, vasopressin, vasoactive intestinal peptide, 25 and circulatory system-borne signaling molecules such as angiotensin, complement, calcitonin, endothelins, formyl-methionyl peptides, glucagon, cholecystokinin and gastrin. NP/VMs can transduce signals directly, modulate the activity or release of other neurotransmitters and hormones, and act as catalytic enzymes in cascades. The effects of NP/VMs range from extremely brief to long-lasting. (Reviewed in Martin, C. R. et al. 30 (1985) Endocrine Physiology, Oxford University Press, New York, NY, pp. 57-62.)

      Regulatory molecules turn individual genes or groups of genes on and off in response to various inductive mechanisms of the cell or organism; act as transcription factors by determining

whether or not transcription is initiated, enhanced, or repressed; and splice transcripts as dictated in a particular cell or tissue. Although they interact with short stretches of DNA scattered throughout the entire genome, most gene expression is regulated near the site at which transcription starts or within the open reading frame of the gene being expressed. Many of the 5 transcription factors incorporate one of a set of DNA-binding structural motifs, each of which contains either  $\alpha$  helices or  $\beta$  sheets and binds to the major groove of DNA. (Pabo, C.O. and R.T. Sauer (1992) *Ann. Rev. Biochem.* 61:1053-95.) Other domains of transcription factors may form crucial contacts with the DNA. In addition, accessory proteins provide important interactions which may convert a particular protein complex to an activator or a repressor or may prevent 10 binding. (Alberts, B. et al. (1994) *Molecular Biology of the Cell*, Garland Publishing Co, New York, NY pp. 401-474.)

The discovery of new human signal peptide-containing proteins and the polynucleotides encoding them satisfies a need in the art by providing new compositions which are useful in the diagnosis, prevention, and treatment of cell proliferative disorders 15 including cancer; inflammation; and cardiovascular, neurological, reproductive, and developmental disorders.

## SUMMARY OF THE INVENTION

The invention features substantially purified polypeptides, proteins with signal 20 peptides, referred to collectively as "HSPP" and individually as "HSPP-1", "HSPP-2", "HSPP-3", "HSPP-4", "HSPP-5", "HSPP-6", "HSPP-7", "HSPP-8", "HSPP-9", "HSPP-10", "HSPP-11", "HSPP-12", "HSPP-13", "HSPP-14", "HSPP-15", "HSPP-16", "HSPP-17", "HSPP-18", "HSPP-19", "HSPP-20", "HSPP-21", "HSPP-22", "HSPP-23", "HSPP-24", "HSPP-25", "HSPP-26", "HSPP-27", "HSPP-28", "HSPP-29", "HSPP-30", "HSPP-25", "HSPP-31", "HSPP-32", "HSPP-33", "HSPP-34", "HSPP-35", "HSPP-36", "HSPP-37", "HSPP-38", "HSPP-39", "HSPP-40", "HSPP-41", "HSPP-42", "HSPP-43", "HSPP-44", "HSPP-45", "HSPP-46", "HSPP-47", "HSPP-48", "HSPP-49", "HSPP-50", "HSPP-51", "HSPP-52", "HSPP-53", "HSPP-54", "HSPP-55", "HSPP-56", "HSPP-57", "HSPP-58", "HSPP-59", "HSPP-60", "HSPP-61", "HSPP-62", "HSPP-63", "HSPP-64", "HSPP-65", "HSPP-66", "HSPP-67", "HSPP-68", "HSPP-69", "HSPP-70", "HSPP-71", "HSPP-72", "HSPP-73", "HSPP-74", "HSPP-75", "HSPP-76", "HSPP-77", "HSPP-78", "HSPP-79", "HSPP-80", "HSPP-81", "HSPP-82", "HSPP-83", "HSPP-84", "HSPP-85", "HSPP-86", "HSPP-87", "HSPP-88", "HSPP-89", "HSPP-90", "HSPP-91", "HSPP-92", "HSPP-93", "HSPP-94", "HSPP-95", "HSPP-96", "HSPP-97", "HSPP-98", "HSPP-99", "HSPP-100", "HSPP-

101", "HSPP-102", "HSPP-103", "HSPP-104", "HSPP-105", "HSPP-106", "HSPP-107",  
"HSPP-108", "HSPP-109", "HSPP-110", "HSPP-111", "HSPP-112", "HSPP-113", "HSPP-  
114", "HSPP-115", "HSPP-116", "HSPP-117", "HSPP-118", "HSPP-119", "HSPP-120",  
5 "HSPP-121", "HSPP-122", "HSPP-123", "HSPP-124", "HSPP-125", "HSPP-126",  
"HSPP-127", "HSPP-128", "HSPP-129", "HSPP-130", "HSPP-131", "HSPP-132",  
"HSPP-133", and "HSPP-134". In one aspect, the invention provides a substantially  
purified polypeptide comprising an amino acid sequence selected from the group  
consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5,  
SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID  
10 NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16,  
SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ  
ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID  
NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, SEQ ID  
NO:32, SEQ ID NO:33, SEQ ID NO:34, SEQ ID NO:35, SEQ ID NO:36, SEQ ID NO:37,  
15 SEQ ID NO:38, SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:41, SEQ ID NO:42, SEQ  
ID NO:43, SEQ ID NO:44, SEQ ID NO:45, SEQ ID NO:46, SEQ ID NO:47, SEQ ID  
NO:48, SEQ ID NO:49, SEQ ID NO:50, SEQ ID NO:51, SEQ ID NO:52, SEQ ID NO:53,  
SEQ ID NO:54, SEQ ID NO:55, SEQ ID NO:56, SEQ ID NO:57, SEQ ID NO:58, SEQ  
ID NO:59, SEQ ID NO:60, SEQ ID NO:61, SEQ ID NO:62, SEQ ID NO:63, SEQ ID  
20 NO:64, SEQ ID NO:65, SEQ ID NO:66, SEQ ID NO:67, SEQ ID NO:68, SEQ ID NO:69,  
SEQ ID NO:70, SEQ ID NO:71, SEQ ID NO:72, SEQ ID NO:73, SEQ ID NO:74, SEQ  
ID NO:75, SEQ ID NO:76, SEQ ID NO:77, SEQ ID NO:78, SEQ ID NO:79, SEQ ID  
NO:80, SEQ ID NO:81, SEQ ID NO:82, SEQ ID NO:83, SEQ ID NO:84, SEQ ID NO:85,  
SEQ ID NO:86, SEQ ID NO:87, SEQ ID NO:88, SEQ ID NO:89, SEQ ID NO:90, SEQ  
25 ID NO:91, SEQ ID NO:92, SEQ ID NO:93, SEQ ID NO:94, SEQ ID NO:95, SEQ ID  
NO:96, SEQ ID NO:97, SEQ ID NO:98, SEQ ID NO:99, SEQ ID NO:100, SEQ ID  
NO:101, SEQ ID NO:102, SEQ ID NO:103, SEQ ID NO:104, SEQ ID NO:105, SEQ ID  
NO:106, SEQ ID NO:107, SEQ ID NO:108, SEQ ID NO:109, SEQ ID NO:110, SEQ ID  
NO:111, SEQ ID NO:112, SEQ ID NO:113, SEQ ID NO:114, SEQ ID NO:115, SEQ ID  
30 NO:116, SEQ ID NO:117, SEQ ID NO:118, SEQ ID NO:119, SEQ ID NO:120, SEQ ID  
NO:121, SEQ ID NO:122, SEQ ID NO:123, SEQ ID NO:124, SEQ ID NO:125, SEQ ID  
NO:126, SEQ ID NO:127, SEQ ID NO:128, SEQ ID NO:129, SEQ ID NO:130, SEQ ID

NO:131, SEQ ID NO:132, SEQ ID NO:133, SEQ ID NO:134 (SEQ ID NO:1-134), and fragments thereof.

The invention further provides a substantially purified variant having at least 90% amino acid identity to at least one of the amino acid sequences selected from the group consisting of SEQ ID NO:1-134, and fragments thereof. The invention also provides an isolated and purified polynucleotide encoding the polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO:1-134, and fragments thereof. The invention also includes an isolated and purified polynucleotide variant having at least 90% polynucleotide sequence identity to the polynucleotide encoding the polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO:1-134, and fragments thereof.

Additionally, the invention provides an isolated and purified polynucleotide which hybridizes under stringent conditions to the polynucleotide encoding the polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO:1-134, and fragments thereof. The invention also provides an isolated and purified polynucleotide having a sequence which is complementary to the polynucleotide encoding the polypeptide comprising the amino acid sequence selected from the group consisting of SEQ ID NO:1-134, and fragments thereof.

The invention also provides an isolated and purified polynucleotide comprising a polynucleotide sequence selected from the group consisting of SEQ ID NO:135, SEQ ID NO:136, SEQ ID NO:137, SEQ ID NO:138, SEQ ID NO:139, SEQ ID NO:140, SEQ ID NO:141, SEQ ID NO:142, SEQ ID NO:143, SEQ ID NO:144, SEQ ID NO:145, SEQ ID NO:146, SEQ ID NO:147, SEQ ID NO:148, SEQ ID NO:149, SEQ ID NO:150, SEQ ID NO:151, SEQ ID NO:152, SEQ ID NO:153, SEQ ID NO:154, SEQ ID NO:155, SEQ ID NO:156, SEQ ID NO:157, SEQ ID NO:158, SEQ ID NO:159, SEQ ID NO:160, SEQ ID NO:161, SEQ ID NO:162, SEQ ID NO:163, SEQ ID NO:164, SEQ ID NO:165, SEQ ID NO:166, SEQ ID NO:167, SEQ ID NO:168, SEQ ID NO:169, SEQ ID NO:170, SEQ ID NO:171, SEQ ID NO:172, SEQ ID NO:173, SEQ ID NO:174, SEQ ID NO:175, SEQ ID NO:176, SEQ ID NO:177, SEQ ID NO:178, SEQ ID NO:179, SEQ ID NO:180, SEQ ID NO:181, SEQ ID NO:182, SEQ ID NO:183, SEQ ID NO:184, SEQ ID NO:185, SEQ ID NO:186, SEQ ID NO:187, SEQ ID NO:188, SEQ ID NO:189, SEQ ID NO:190, SEQ ID NO:191, SEQ ID NO:192, SEQ ID NO:193, SEQ ID NO:194, SEQ ID NO:195, SEQ ID

NO:196, SEQ ID NO:197, SEQ ID NO:198, SEQ ID NO:199, SEQ ID NO:200, SEQ ID NO:201, SEQ ID NO:202, SEQ ID NO:203, SEQ ID NO:204, SEQ ID NO:205, SEQ ID NO:206, SEQ ID NO:207, SEQ ID NO:208, SEQ ID NO:209, SEQ ID NO:210, SEQ ID NO:211, SEQ ID NO:212, SEQ ID NO:213, SEQ ID NO:214, SEQ ID NO:215, SEQ ID NO:216, SEQ ID NO:217, SEQ ID NO:218, SEQ ID NO:219, SEQ ID NO:220, SEQ ID NO:221, SEQ ID NO:222, SEQ ID NO:223, SEQ ID NO:224, SEQ ID NO:225, SEQ ID NO:226, SEQ ID NO:227, SEQ ID NO:228, SEQ ID NO:229, SEQ ID NO:230, SEQ ID NO:231, SEQ ID NO:232, SEQ ID NO:233, SEQ ID NO:234, SEQ ID NO:235, SEQ ID NO:236, SEQ ID NO:237, SEQ ID NO:238, SEQ ID NO:239, SEQ ID NO:240, SEQ ID NO:241, SEQ ID NO:242, SEQ ID NO:243, SEQ ID NO:244, SEQ ID NO:245, SEQ ID NO:246, SEQ ID NO:247, SEQ ID NO:248, SEQ ID NO:249, SEQ ID NO:250, SEQ ID NO:251, SEQ ID NO:252, SEQ ID NO:253, SEQ ID NO:254, SEQ ID NO:255, SEQ ID NO:256, SEQ ID NO:257, SEQ ID NO:258, SEQ ID NO:259, SEQ ID NO:260, SEQ ID NO:261, SEQ ID NO:262, SEQ ID NO:263, SEQ ID NO:264, SEQ ID NO:265, SEQ ID NO:266, SEQ ID NO:267, SEQ ID NO:268 (SEQ ID NO:135-268), and fragments thereof. The invention further provides an isolated and purified polynucleotide variant having at least 90% polynucleotide sequence identity to the polynucleotide sequence selected from the group consisting of SEQ ID NO:135-268, and fragments thereof. The invention also provides an isolated and purified polynucleotide having a sequence which is complementary to the polynucleotide comprising a polynucleotide sequence selected from the group consisting of SEQ ID NO:135-268, and fragments thereof.

The invention also provides a method for detecting a polynucleotide in a sample containing nucleic acids, the method comprising the steps of (a) hybridizing the complement of the polynucleotide sequence to at least one of the polynucleotides of the sample, thereby forming a hybridization complex; and (b) detecting the hybridization complex, wherein the presence of the hybridization complex correlates with the presence of a polynucleotide in the sample. In one aspect, the method further comprises amplifying the polynucleotide prior to hybridization.

The invention further provides an expression vector containing at least a fragment of the polynucleotide encoding the polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO:1-134, and fragments thereof. In another aspect, the expression vector is contained within a host cell.

The invention also provides a method for producing a polypeptide, the method comprising the steps of: (a) culturing the host cell containing an expression vector containing at least a fragment of a polynucleotide under conditions suitable for the expression of the polypeptide; and (b) recovering the polypeptide from the host cell

5 culture.

The invention also provides a pharmaceutical composition comprising a substantially purified polypeptide having the amino acid sequence selected from the group consisting of SEQ ID NO:1-134, and fragments thereof, in conjunction with a suitable pharmaceutical carrier.

10 The invention further includes a purified antibody which binds to a polypeptide selected from the group consisting of SEQ ID NO:1-134, and fragments thereof. The invention also provides a purified agonist and a purified antagonist to the polypeptide.

15 The invention also provides a method for treating or preventing a disorder associated with decreased expression or activity of HSPP, the method comprising administering to a subject in need of such treatment an effective amount of a pharmaceutical composition comprising a substantially purified polypeptide having the amino acid sequence selected from the group consisting of SEQ ID NO:1-134, and fragments thereof, in conjunction with a suitable pharmaceutical carrier.

20 The invention also provides a method for treating or preventing a disorder associated with increased expression or activity of HSPP, the method comprising administering to a subject in need of such treatment an effective amount of an antagonist of a polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NO:1-134, and fragments thereof.

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#### BRIEF DESCRIPTION OF THE TABLE

Table 1 shows nucleotide and polypeptide sequence identification numbers (SEQ ID NO), clone identification numbers (clone ID), cDNA libraries, and cDNA fragments used to assemble full-length sequences encoding HSPP.

30 Table 2 shows features of each polypeptide sequence, including predicted signal peptide sequences, and methods and algorithms used for identification of HSPP.

Table 3 shows the tissue-specific expression patterns of each nucleic acid sequence as determined by northern analysis, diseases, disorders, or conditions associated with these tissues, and the vector into which each cDNA was cloned.

Table 4 describes the tissues used to construct the cDNA libraries from which 5 Incyte cDNA clones encoding HSPP were isolated.

Table 5 shows the programs, their descriptions, references, and threshold parameters used to analyze HSPP.

Table 6 shows the regions of the full-length nucleotide sequences of HSPP to which cDNA fragments of Table 1 correspond.

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### DESCRIPTION OF THE INVENTION

Before the present proteins, nucleotide sequences, and methods are described, it is understood that this invention is not limited to the particular machines, materials and methods described, as these may vary. It is also to be understood that the terminology 15 used herein is for the purpose of describing particular embodiments only, and is not intended to limit the scope of the present invention which will be limited only by the appended claims.

It must be noted that as used herein and in the appended claims, the singular forms "a," "an," and "the" include plural reference unless the context clearly dictates otherwise.

20 Thus, for example, a reference to "a host cell" includes a plurality of such host cells, and a reference to "an antibody" is a reference to one or more antibodies and equivalents thereof known to those skilled in the art, and so forth.

Unless defined otherwise, all technical and scientific terms used herein have the same meanings as commonly understood by one of ordinary skill in the art to which this 25 invention belongs. Although any machines, materials, and methods similar or equivalent to those described herein can be used to practice or test the present invention, the preferred machines, materials and methods are now described. All publications mentioned herein are cited for the purpose of describing and disclosing the cell lines, protocols, reagents and vectors which are reported in the publications and which might be used in connection with 30 the invention. Nothing herein is to be construed as an admission that the invention is not entitled to antedate such disclosure by virtue of prior invention.

## DEFINITIONS

"HSPP" refers to the amino acid sequences of substantially purified HSPP obtained from any species, particularly a mammalian species, including bovine, ovine, porcine, murine, equine, and preferably the human species, from any source, whether natural, 5 synthetic, semi-synthetic, or recombinant.

The term "agonist" refers to a molecule which, when bound to HSPP, increases or prolongs the duration of the effect of HSPP. Agonists may include proteins, nucleic acids, carbohydrates, or any other molecules which bind to and modulate the effect of HSPP.

An "allelic variant" is an alternative form of the gene encoding HSPP. Allelic 10 variants may result from at least one mutation in the nucleic acid sequence and may result in altered mRNAs or in polypeptides whose structure or function may or may not be altered. Any given natural or recombinant gene may have none, one, or many allelic forms. Common mutational changes which give rise to allelic variants are generally ascribed to natural deletions, additions, or substitutions of nucleotides. Each of these 15 types of changes may occur alone, or in combination with the others, one or more times in a given sequence.

"Altered" nucleic acid sequences encoding HSPP include those sequences with deletions, insertions, or substitutions of different nucleotides, resulting in a polynucleotide the same as HSPP or a polypeptide with at least one functional characteristic of HSPP.

20 Included within this definition are polymorphisms which may or may not be readily detectable using a particular oligonucleotide probe of the polynucleotide encoding HSPP, and improper or unexpected hybridization to allelic variants, with a locus other than the normal chromosomal locus for the polynucleotide sequence encoding HSPP. The encoded protein may also be "altered," and may contain deletions, insertions, or substitutions of 25 amino acid residues which produce a silent change and result in a functionally equivalent HSPP. Deliberate amino acid substitutions may be made on the basis of similarity in polarity, charge, solubility, hydrophobicity, hydrophilicity, and/or the amphipathic nature of the residues, as long as the biological or immunological activity of HSPP is retained. For example, negatively charged amino acids may include aspartic acid and glutamic acid, 30 positively charged amino acids may include lysine and arginine, and amino acids with uncharged polar head groups having similar hydrophilicity values may include leucine,

isoleucine, and valine; glycine and alanine; asparagine and glutamine; serine and threonine; and phenylalanine and tyrosine.

The terms "amino acid" or "amino acid sequence" refer to an oligopeptide, peptide, polypeptide, or protein sequence, or a fragment of any of these, and to naturally occurring or synthetic molecules. In this context, "fragments," "immunogenic fragments," or "antigenic fragments" refer to fragments of HSPP which are preferably at least 5 to about 15 amino acids in length, most preferably at least 14 amino acids, and which retain some biological activity or immunological activity of HSPP. Where "amino acid sequence" is recited to refer to an amino acid sequence of a naturally occurring protein molecule, 10 "amino acid sequence" and like terms are not meant to limit the amino acid sequence to the complete native amino acid sequence associated with the recited protein molecule.

"Amplification" relates to the production of additional copies of a nucleic acid sequence. Amplification is generally carried out using polymerase chain reaction (PCR) technologies well known in the art.

15 The term "antagonist" refers to a molecule which, when bound to HSPP, decreases the amount or the duration of the effect of the biological or immunological activity of HSPP. Antagonists may include proteins, nucleic acids, carbohydrates, antibodies, or any other molecules which decrease the effect of HSPP.

The term "antibody" refers to intact molecules as well as to fragments thereof, such 20 as Fab, F(ab')<sub>2</sub>, and Fv fragments, which are capable of binding the epitopic determinant. Antibodies that bind HSPP polypeptides can be prepared using intact polypeptides or using fragments containing small peptides of interest as the immunizing antigen. The polypeptide or oligopeptide used to immunize an animal (e.g., a mouse, a rat, or a rabbit) can be derived from the translation of RNA, or synthesized chemically, and can be 25 conjugated to a carrier protein if desired. Commonly used carriers that are chemically coupled to peptides include bovine serum albumin, thyroglobulin, and keyhole limpet hemocyanin (KLH). The coupled peptide is then used to immunize the animal.

The term "antigenic determinant" refers to that fragment of a molecule (i.e., an epitope) that makes contact with a particular antibody. When a protein or a fragment of a 30 protein is used to immunize a host animal, numerous regions of the protein may induce the production of antibodies which bind specifically to antigenic determinants (given regions or three-dimensional structures on the protein). An antigenic determinant may compete

with the intact antigen (i.e., the immunogen used to elicit the immune response) for binding to an antibody.

The term "antisense" refers to any composition containing a nucleic acid sequence which is complementary to the "sense" strand of a specific nucleic acid sequence.

5 Antisense molecules may be produced by any method including synthesis or transcription. Once introduced into a cell, the complementary nucleotides combine with natural sequences produced by the cell to form duplexes and to block either transcription or translation. The designation "negative" can refer to the antisense strand, and the designation "positive" can refer to the sense strand.

10 The term "biologically active," refers to a protein having structural, regulatory, or biochemical functions of a naturally occurring molecule. Likewise, "immunologically active" refers to the capability of the natural, recombinant, or synthetic HSPP, or of any oligopeptide thereof, to induce a specific immune response in appropriate animals or cells and to bind with specific antibodies.

15 The terms "complementary" or "complementarity" refer to the natural binding of polynucleotides by base pairing. For example, the sequence "5' A-G-T 3'" bonds to the complementary sequence "3' T-C-A 5'." Complementarity between two single-stranded molecules may be "partial," such that only some of the nucleic acids bind, or it may be "complete," such that total complementarity exists between the single stranded molecules.

20 The degree of complementarity between nucleic acid strands has significant effects on the efficiency and strength of the hybridization between the nucleic acid strands. This is of particular importance in amplification reactions, which depend upon binding between nucleic acids strands, and in the design and use of peptide nucleic acid (PNA) molecules.

A "composition comprising a given polynucleotide sequence" or a "composition

25 comprising a given amino acid sequence" refer broadly to any composition containing the given polynucleotide or amino acid sequence. The composition may comprise a dry formulation or an aqueous solution. Compositions comprising polynucleotide sequences encoding HSPP or fragments of HSPP may be employed as hybridization probes. The probes may be stored in freeze-dried form and may be associated with a stabilizing agent

30 such as a carbohydrate. In hybridizations, the probe may be deployed in an aqueous solution containing salts (e.g., NaCl), detergents (e.g., sodium dodecyl sulfate; SDS), and other components (e.g., Denhardt's solution, dry milk, salmon sperm DNA, etc.).

“Consensus sequence” refers to a nucleic acid sequence which has been resequenced to resolve uncalled bases, extended using XL-PCR kit (Perkin-Elmer, Norwalk CT) in the 5' and/or the 3' direction, and resequenced, or which has been assembled from the overlapping sequences of more than one Incyte Clone using a 5 computer program for fragment assembly, such as the GELVIEW Fragment Assembly system (GCG, Madison WI). Some sequences have been both extended and assembled to produce the consensus sequence.

10 The term “correlates with expression of a polynucleotide” indicates that the detection of the presence of nucleic acids, the same or related to a nucleic acid sequence encoding HSPP, by northern analysis is indicative of the presence of nucleic acids encoding HSPP in a sample, and thereby correlates with expression of the transcript from the polynucleotide encoding HSPP.

15 A “deletion” refers to a change in the amino acid or nucleotide sequence that results in the absence of one or more amino acid residues or nucleotides.

20 The term “derivative” refers to the chemical modification of a polypeptide sequence, or a polynucleotide sequence. Chemical modifications of a polynucleotide sequence can include, for example, replacement of hydrogen by an alkyl, acyl, or amino group. A derivative polynucleotide encodes a polypeptide which retains at least one biological or immunological function of the natural molecule. A derivative polypeptide is 25 one modified by glycosylation, pegylation, or any similar process that retains at least one biological or immunological function of the polypeptide from which it was derived.

25 The term “similarity” refers to a degree of complementarity. There may be partial similarity or complete similarity. The word “identity” may substitute for the word “similarity.” A partially complementary sequence that at least partially inhibits an identical sequence from hybridizing to a target nucleic acid is referred to as “substantially similar.” The inhibition of hybridization of the completely complementary sequence to the target sequence may be examined using a hybridization assay (Southern or northern blot, solution hybridization, and the like) under conditions of reduced stringency. A substantially similar sequence or hybridization probe will compete for and inhibit the 30 binding of a completely similar (identical) sequence to the target sequence under conditions of reduced stringency. This is not to say that conditions of reduced stringency are such that non-specific binding is permitted, as reduced stringency conditions require

that the binding of two sequences to one another be a specific (i.e., a selective) interaction. The absence of non-specific binding may be tested by the use of a second target sequence which lacks even a partial degree of complementarity (e.g., less than about 30% similarity or identity). In the absence of non-specific binding, the substantially similar sequence or 5 probe will not hybridize to the second non-complementary target sequence.

The phrases "percent identity" or "% identity" refer to the percentage of sequence similarity found in a comparison of two or more amino acid or nucleic acid sequences. Percent identity can be determined electronically, e.g., by using the MEGALIGN program (DNASTAR, Madison WI) which creates alignments between two or more sequences 10 according to methods selected by the user, e.g., the clustal method. (See, e.g., Higgins, D.G. and P.M. Sharp (1988) Gene 73:237-244.) The clustal algorithm groups sequences into clusters by examining the distances between all pairs. The clusters are aligned pairwise and then in groups. The percentage similarity between two amino acid sequences, e.g., sequence A and sequence B, is calculated by dividing the length of 15 sequence A, minus the number of gap residues in sequence A, minus the number of gap residues in sequence B, into the sum of the residue matches between sequence A and sequence B, times one hundred. Gaps of low or of no similarity between the two amino acid sequences are not included in determining percentage similarity. Percent identity between nucleic acid sequences can also be counted or calculated by other methods known 20 in the art, e.g., the Jotun Hein method. (See, e.g., Hein, J. (1990) Methods Enzymol. 183:626-645.) Identity between sequences can also be determined by other methods known in the art, e.g., by varying hybridization conditions.

"Human artificial chromosomes" (HACs) are linear microchromosomes which may contain DNA sequences of about 6 kb to 10 Mb in size, and which contain all of the 25 elements required for stable mitotic chromosome segregation and maintenance.

The term "humanized antibody" refers to antibody molecules in which the amino acid sequence in the non-antigen binding regions has been altered so that the antibody more closely resembles a human antibody, and still retains its original binding ability.

"Hybridization" refers to any process by which a strand of nucleic acid binds with 30 a complementary strand through base pairing.

The term "hybridization complex" refers to a complex formed between two nucleic acid sequences by virtue of the formation of hydrogen bonds between complementary

bases. A hybridization complex may be formed in solution (e.g., C<sub>0</sub>t or R<sub>0</sub>t analysis) or formed between one nucleic acid sequence present in solution and another nucleic acid sequence immobilized on a solid support (e.g., paper, membranes, filters, chips, pins or glass slides, or any other appropriate substrate to which cells or their nucleic acids have 5 been fixed).

The words "insertion" or "addition" refer to changes in an amino acid or nucleotide sequence resulting in the addition of one or more amino acid residues or nucleotides, respectively, to the sequence found in the naturally occurring molecule.

"Immune response" can refer to conditions associated with inflammation, trauma, 10 immune disorders, or infectious or genetic disease, etc. These conditions can be characterized by expression of various factors, e.g., cytokines, chemokines, and other signaling molecules, which may affect cellular and systemic defense systems.

The term "microarray" refers to an arrangement of distinct polynucleotides on a substrate.

15 The terms "element" or "array element" in a microarray context, refer to hybridizable polynucleotides arranged on the surface of a substrate.

The term "modulate" refers to a change in the activity of HSPP. For example, modulation may cause an increase or a decrease in protein activity, binding characteristics, or any other biological, functional, or immunological properties of HSPP.

20 The phrases "nucleic acid" or "nucleic acid sequence," as used herein, refer to a nucleotide, oligonucleotide, polynucleotide, or any fragment thereof. These phrases also refer to DNA or RNA of genomic or synthetic origin which may be single-stranded or double-stranded and may represent the sense or the antisense strand, to peptide nucleic acid (PNA), or to any DNA-like or RNA-like material. In this context, "fragments" refers 25 to those nucleic acid sequences which, comprise a region of unique polynucleotide sequence that specifically identifies SEQ ID NO:135-268, for example, as distinct from any other sequence in the same genome. For example, a fragment of SEQ ID NO:135-268 is useful in hybridization and amplification technologies and in analogous methods that distinguish SEQ ID NO:135-268 from related polynucleotide sequences. A fragment of 30 SEQ ID NO:135-268 is at least about 15-20 nucleotides in length. The precise length of the fragment of SEQ ID NO:135-268 and the region of SEQ ID NO:135-268 to which the fragment corresponds are routinely determinable by one of ordinary skill in the art based

on the intended purpose for the fragment. In some cases, a fragment, when translated, would produce polypeptides retaining some functional characteristic, e.g., antigenicity, or structural domain characteristic, e.g., ATP-binding site, of the full-length polypeptide.

The terms "operably associated" or "operably linked" refer to functionally related 5 nucleic acid sequences. A promoter is operably associated or operably linked with a coding sequence if the promoter controls the translation of the encoded polypeptide. While operably associated or operably linked nucleic acid sequences can be contiguous and in the same reading frame, certain genetic elements, e.g., repressor genes, are not contiguously linked to the sequence encoding the polypeptide but still bind to operator 10 sequences that control expression of the polypeptide.

The term "oligonucleotide" refers to a nucleic acid sequence of at least about 6 nucleotides to 60 nucleotides, preferably about 15 to 30 nucleotides, and most preferably about 20 to 25 nucleotides, which can be used in PCR amplification or in a hybridization assay or microarray. "Oligonucleotide" is substantially equivalent to the terms

15 "amplimer," "primer," "oligomer," and "probe," as these terms are commonly defined in the art.

"Peptide nucleic acid" (PNA) refers to an antisense molecule or anti-gene agent which comprises an oligonucleotide of at least about 5 nucleotides in length linked to a peptide backbone of amino acid residues ending in lysine. The terminal lysine confers 20 solubility to the composition. PNAs preferentially bind complementary single stranded DNA or RNA and stop transcript elongation, and may be pegylated to extend their lifespan in the cell.

The term "sample" is used in its broadest sense. A sample suspected of containing 25 nucleic acids encoding HSPP, or fragments thereof, or HSPP itself, may comprise a bodily fluid; an extract from a cell, chromosome, organelle, or membrane isolated from a cell; a cell; genomic DNA, RNA, or cDNA, in solution or bound to a substrate; a tissue; a tissue print; etc.

The terms "specific binding" or "specifically binding" refer to that interaction 30 between a protein or peptide and an agonist, an antibody, or an antagonist. The interaction is dependent upon the presence of a particular structure of the protein, e.g., the antigenic determinant or epitope, recognized by the binding molecule. For example, if an antibody is specific for epitope "A," the presence of a polypeptide containing the epitope A, or the

presence of free unlabeled A, in a reaction containing free labeled A and the antibody will reduce the amount of labeled A that binds to the antibody.

The term "stringent conditions" refers to conditions which permit hybridization between polynucleotides and the claimed polynucleotides. Stringent conditions can be 5 defined by salt concentration, the concentration of organic solvent, e.g., formamide, temperature, and other conditions well known in the art. In particular, stringency can be increased by reducing the concentration of salt, increasing the concentration of formamide, or raising the hybridization temperature.

The term "substantially purified" refers to nucleic acid or amino acid sequences 10 that are removed from their natural environment and are isolated or separated, and are at least about 60% free, preferably about 75% free, and most preferably about 90% free from other components with which they are naturally associated.

A "substitution" refers to the replacement of one or more amino acids or nucleotides by different amino acids or nucleotides, respectively.

15 "Substrate" refers to any suitable rigid or semi-rigid support including membranes, filters, chips, slides, wafers, fibers, magnetic or nonmagnetic beads, gels, tubing, plates, polymers, microparticles and capillaries. The substrate can have a variety of surface forms, such as wells, trenches, pins, channels and pores, to which polynucleotides or polypeptides are bound.

20 "Transformation" describes a process by which exogenous DNA enters and changes a recipient cell. Transformation may occur under natural or artificial conditions according to various methods well known in the art, and may rely on any known method for the insertion of foreign nucleic acid sequences into a prokaryotic or eukaryotic host cell. The method for transformation is selected based on the type of host cell being 25 transformed and may include, but is not limited to, viral infection, electroporation, heat shock, lipofection, and particle bombardment. The term "transformed" cells includes stably transformed cells in which the inserted DNA is capable of replication either as an autonomously replicating plasmid or as part of the host chromosome, as well as transiently transformed cells which express the inserted DNA or RNA for limited periods of time.

30 A "variant" of HSPP polypeptides refers to an amino acid sequence that is altered by one or more amino acid residues. The variant may have "conservative" changes, wherein a substituted amino acid has similar structural or chemical properties (e.g.,

replacement of leucine with isoleucine). More rarely, a variant may have "nonconservative" changes (e.g., replacement of glycine with tryptophan). Analogous minor variations may also include amino acid deletions or insertions, or both. Guidance in determining which amino acid residues may be substituted, inserted, or deleted without 5 abolishing biological or immunological activity may be found using computer programs well known in the art, for example, LASERGENE software (DNASTAR).

The term "variant," when used in the context of a polynucleotide sequence, may encompass a polynucleotide sequence related to HSPP. This definition may also include, for example, "allelic" (as defined above), "splice," "species," or "polymorphic" variants. 10 A splice variant may have significant identity to a reference molecule, but will generally have a greater or lesser number of polynucleotides due to alternate splicing of exons during mRNA processing. The corresponding polypeptide may possess additional functional domains or an absence of domains. Species variants are polynucleotide sequences that vary from one species to another. The resulting polypeptides generally will 15 have significant amino acid identity relative to each other. A polymorphic variant is a variation in the polynucleotide sequence of a particular gene between individuals of a given species. Polymorphic variants also may encompass "single nucleotide polymorphisms" (SNPs) in which the polynucleotide sequence varies by one base. The presence of SNPs may be indicative of, for example, a certain population, a disease state, 20 or a propensity for a disease state.

## THE INVENTION

The invention is based on the discovery of new human signal peptide-containing proteins (HSPP), the polynucleotides encoding HSPP, and the use of these compositions 25 for the diagnosis, treatment, or prevention of cell proliferative disorders including cancer, inflammation; and cardiovascular, neurological, reproductive, and developmental disorders.

Table 1 lists the Incyte Clones used to derive full length nucleotide sequences encoding HSPP. Columns 1 and 2 show the sequence identification numbers (SEQ ID 30 NO) of the amino acid and nucleic acid sequences, respectively. Column 3 shows the Clone ID of the Incyte Clone in which nucleic acids encoding each HSPP were identified, and column 4, the cDNA libraries from which these clones were isolated. Column 5

shows Incyte clones, their corresponding cDNA libraries, and shotgun sequences. The clones and shotgun sequences are part of the consensus nucleotide sequence of each HSPP and are useful as fragments in hybridization technologies.

Table 6 shows the regions of the full-length nucleotide sequences of HSPP to 5 which cDNA fragments of Table 1 correspond. Column 1 lists nucleotide sequence identifiers and column 2 shows the clone ID of the Incyte clone in which nucleic acids encoding each HSPP were identified. Column 3 shows Incyte clones and shotgun sequences which are part of the consensus nucleotide sequence of each HSPP and are useful as fragments in hybridization technologies. Column 4 lists the starting nucleotide 10 position and column 5 the ending nucleotide position of the region of the full-length HSPP to which the cDNA fragment corresponds.

The columns of Table 2 show various properties of the polypeptides of the invention: column 1 references the SEQ ID NO; column 2 shows the number of amino acid residues in each polypeptide; column 3, potential phosphorylation sites; column 4, 15 potential glycosylation sites; column 5, the amino acid residues comprising signature sequences and motifs; column 6, the identity of each protein; and column 7, analytical methods used to identify each HSPP as a signal peptide-containing protein. Note that in column 5, the first line of each cell lists the amino acid residues comprising predicted signal peptide sequences. Additional identifying motifs or signatures are also listed in 20 column 5. Of particular note is the presence of a glycosyl hydrolase family 9 active site signature in SEQ ID NO:126, a ribosomal protein S18 signature in SEQ ID NO:127, an adrenodoxin family iron-sulfur binding region signature and a cytochrome c family heme-binding site signature in SEQ ID NO:132, and a urotensin II signature sequence in SEQ ID NO:96.

25 Using BLAST, SEQ ID NO:68 (HSPP-68) has been identified as a TWIK-related acid-sensitive K<sup>+</sup> channel, and SEQ ID NO:92 (HSPP-92) has been identified as a tyrosine-specific protein phosphatase. The tyrosine-specific protein phosphatases signature in SEQ ID NO:92 (HSPP-92) from about V328 through about F340 (including the putative active site cysteine residue at C330) was identified using BLOCKS and 30 PRINTS. Also of note is the identification of SEQ ID NO:66 (HSPP-66) as a steroid binding protein using BLAST.

The columns of Table 3 show the tissue-specificity and diseases, disorders, or conditions associated with nucleotide sequences encoding HSPP. The first column of Table 3 lists the nucleotide sequence identifiers. The second column lists tissue categories which express HSPP as a fraction of total tissue categories expressing HSPP. The third 5 column lists the diseases, disorders, or conditions associated with those tissues expressing HSPP. The fourth column lists the vectors used to subclone the cDNA library. Of particular note is the expression of SEQ ID NO:200, SEQ ID NO:203, and SEQ ID NO:225 in lung tissues; the expression of SEQ ID NO:212, SEQ ID NO:216, and SEQ ID NO:220 in reproductive tissues; the expression of SEQ ID NO:223 in cancerous tissues; 10 the expression of SEQ ID NO:232 in gastrointestinal tissue, specifically the small intestine or colon (fifteen out of sixteen (93.8%) cDNA libraries); and the expression of SEQ ID NO:224 in cancerous and proliferating tissues. Also of particular interest is the tissue-specific expression of SEQ ID NO:252 and SEQ ID NO:257. SEQ ID NO:252 is derived from OVARTUT01, an ovarian tumor cDNA library and is exclusively expressed in 15 reproductive tumor tissue. SEQ ID NO:257 is derived from THP1AZT01, a 5-aza-2'-deoxycytidine treated human promonocyte cDNA library and is exclusively expressed in hematopoietic tissue.

The following fragments of the nucleotide sequences encoding HSPP are useful in hybridization or amplification technologies to identify SEQ ID NO:135-268 and to 20 distinguish between SEQ ID NO:135-268 and related polynucleotide sequences. The useful fragments are the fragment of SEQ ID NO:230 from about nucleotide 75 to about nucleotide 104; the fragment of SEQ ID NO:231 from about nucleotide 210 to about nucleotide 239; the fragment of SEQ ID NO:232 from about nucleotide 157 to about nucleotide 186; the fragment of SEQ ID NO:233 from about nucleotide 268 to about 25 nucleotide 297; the fragment of SEQ ID NO:234 from about nucleotide 160 to about nucleotide 186; the fragment of SEQ ID NO:235 from about nucleotide 201 to about nucleotide 230; the fragment of SEQ ID NO:236 from about nucleotide 165 to about nucleotide 194; the fragment of SEQ ID NO:237 from about nucleotide 366 to about nucleotide 395; the fragment of SEQ ID NO:238 from about nucleotide 714 to about 30 nucleotide 743; the fragment of SEQ ID NO:239 from about nucleotide 1731 to about nucleotide 1760; the fragment of SEQ ID NO:240 from about nucleotide 419 to about nucleotide 448; the fragment of SEQ ID NO:241 from about nucleotide 494 to about

nucleotide 523; the fragment of SEQ ID NO:242 from about nucleotide 100 to about nucleotide 129; the fragment of SEQ ID NO:243 from about nucleotide 104 to about nucleotide 133; the fragment of SEQ ID NO:244 from about nucleotide 136 to about nucleotide 165; the fragment of SEQ ID NO:245 from about nucleotide 140 to about 5 nucleotide 169; the fragment of SEQ ID NO:246 from about nucleotide 125 to about nucleotide 154; the fragment of SEQ ID NO:247 from about nucleotide 687 to about nucleotide 758; the fragment of SEQ ID NO:248 from about nucleotide 327 to about nucleotide 398; the fragment of SEQ ID NO:249 from about nucleotide 741 to about nucleotide 785; the fragment of SEQ ID NO:250 from about nucleotide 184 to about 10 nucleotide 255; the fragment of SEQ ID NO:251 from about nucleotide 165 to about nucleotide 242; the fragment of SEQ ID NO:252 from about nucleotide 271 to about nucleotide 342; the fragment of SEQ ID NO:253 from about nucleotide 1081 to about nucleotide 1152; the fragment of SEQ ID NO:254 from about nucleotide 781 to about nucleotide 852; the fragment of SEQ ID NO:255 from about nucleotide 620 to about 15 nucleotide 691; the fragment of SEQ ID NO:256 from about nucleotide 872 to about nucleotide 916; the fragment of SEQ ID NO:257 from about nucleotide 242 to about nucleotide 313; the fragment of SEQ ID NO:258 from about nucleotide 595 to about nucleotide 648; the fragment of SEQ ID NO:259 from about nucleotide 163 to about nucleotide 216; the fragment of SEQ ID NO:260 from about nucleotide 244 to about 20 nucleotide 315; the fragment of SEQ ID NO:261 from about nucleotide 75 to about nucleotide 128; the fragment of SEQ ID NO:262 from about nucleotide 650 to about nucleotide 703; the fragment of SEQ ID NO:263 from about nucleotide 143 to about nucleotide 214; the fragment of SEQ ID NO:264 from about nucleotide 434 to about nucleotide 487; the fragment of SEQ ID NO:265 from about nucleotide 218 to about 25 nucleotide 271; the fragment of SEQ ID NO:266 from about nucleotide 89 to about nucleotide 145; the fragment of SEQ ID NO:267 from about nucleotide 198 to about nucleotide 254; and the fragment of SEQ ID NO:268 from about nucleotide 10 to about nucleotide 54.

The invention also encompasses HSPP variants. A preferred HSPP variant is one 30 which has at least about 80%, more preferably at least about 90%, and most preferably at least about 95% amino acid sequence identity to the HSPP amino acid sequence, and which contains at least one functional or structural characteristic of HSPP.

The invention also encompasses polynucleotides which encode HSPP. In a particular embodiment, the invention encompasses a polynucleotide sequence comprising a sequence selected from the group consisting of SEQ ID NO:135-268, which encodes HSPP.

5 The invention also encompasses a variant of a polynucleotide sequence encoding HSPP. In particular, such a variant polynucleotide sequence will have at least about 80%, more preferably at least about 90%, and most preferably at least about 95% polynucleotide sequence identity to the polynucleotide sequence encoding HSPP. A particular aspect of the invention encompasses a variant of a polynucleotide sequence comprising a sequence 10 selected from the group consisting of SEQ ID NO:135-268 which has at least about 80%, more preferably at least about 90%, and most preferably at least about 95% polynucleotide sequence identity to a nucleic acid sequence selected from the group consisting of SEQ ID NO:135-268. Any one of the polynucleotide variants described above can encode an amino acid sequence which contains at least one functional or structural characteristic of 15 HSPP.

It will be appreciated by those skilled in the art that as a result of the degeneracy of the genetic code, a multitude of polynucleotide sequences encoding HSPP, some bearing minimal similarity to the polynucleotide sequences of any known and naturally occurring gene, may be produced. Thus, the invention contemplates each and every possible 20 variation of polynucleotide sequence that could be made by selecting combinations based on possible codon choices. These combinations are made in accordance with the standard triplet genetic code as applied to the polynucleotide sequence of naturally occurring HSPP, and all such variations are to be considered as being specifically disclosed.

Although nucleotide sequences which encode HSPP and its variants are preferably 25 capable of hybridizing to the nucleotide sequence of the naturally occurring HSPP under appropriately selected conditions of stringency, it may be advantageous to produce nucleotide sequences encoding HSPP or its derivatives possessing a substantially different codon usage, e.g., inclusion of non-naturally occurring codons. Codons may be selected to increase the rate at which expression of the peptide occurs in a particular prokaryotic or 30 eukaryotic host in accordance with the frequency with which particular codons are utilized by the host. Other reasons for substantially altering the nucleotide sequence encoding HSPP and its derivatives without altering the encoded amino acid sequences include the

production of RNA transcripts having more desirable properties, such as a greater half-life, than transcripts produced from the naturally occurring sequence.

The invention also encompasses production of DNA sequences which encode HSPP and HSPP derivatives, or fragments thereof, entirely by synthetic chemistry. After 5 production, the synthetic sequence may be inserted into any of the many available expression vectors and cell systems using reagents well known in the art. Moreover, synthetic chemistry may be used to introduce mutations into a sequence encoding HSPP or any fragment thereof.

Also encompassed by the invention are polynucleotide sequences that are capable 10 of hybridizing to the claimed polynucleotide sequences, and, in particular, to those shown in SEQ ID NO:135-268 and fragments thereof under various conditions of stringency. (See, e.g., Wahl, G.M. and S.L. Berger (1987) *Methods Enzymol.* 152:399-407; Kimmel, A.R. (1987) *Methods Enzymol.* 152:507-511.) For example, stringent salt concentration will ordinarily be less than about 750 mM NaCl and 75 mM trisodium citrate, preferably 15 less than about 500 mM NaCl and 50 mM trisodium citrate, and most preferably less than about 250 mM NaCl and 25 mM trisodium citrate. Low stringency hybridization can be obtained in the absence of organic solvent, e.g., formamide, while high stringency hybridization can be obtained in the presence of at least about 35% formamide, and most preferably at least about 50% formamide. Stringent temperature conditions will ordinarily 20 include temperatures of at least about 30°C, more preferably of at least about 37°C, and most preferably of at least about 42°C. Varying additional parameters, such as hybridization time, the concentration of detergent, e.g., sodium dodecyl sulfate (SDS), and the inclusion or exclusion of carrier DNA, are well known to those skilled in the art. Various levels of stringency are accomplished by combining these various conditions as 25 needed. In a preferred embodiment, hybridization will occur at 30°C in 750 mM NaCl, 75 mM trisodium citrate, and 1% SDS. In a more preferred embodiment, hybridization will occur at 37°C in 500 mM NaCl, 50 mM trisodium citrate, 1% SDS, 35% formamide, and 100 µg/ml denatured salmon sperm DNA (ssDNA). In a most preferred embodiment, hybridization will occur at 42°C in 250 mM NaCl, 25 mM trisodium citrate, 1% SDS, 50 30 % formamide, and 200 µg/ml ssDNA. Useful variations on these conditions will be readily apparent to those skilled in the art.

The washing steps which follow hybridization can also vary in stringency. Wash stringency conditions can be defined by salt concentration and by temperature. As above, wash stringency can be increased by decreasing salt concentration or by increasing temperature. For example, stringent salt concentration for the wash steps will preferably

5 be less than about 30 mM NaCl and 3 mM trisodium citrate, and most preferably less than about 15 mM NaCl and 1.5 mM trisodium citrate. Stringent temperature conditions for the wash steps will ordinarily include temperature of at least about 25°C, more preferably of at least about 42°C, and most preferably of at least about 68°C. In a preferred embodiment, wash steps will occur at 25°C in 30 mM NaCl, 3 mM trisodium citrate, and

10 0.1% SDS. In a more preferred embodiment, wash steps will occur at 42°C in 15 mM NaCl, 1.5 mM trisodium citrate, and 0.1% SDS. In a most preferred embodiment, wash steps will occur at 68°C in 15 mM NaCl, 1.5 mM trisodium citrate, and 0.1% SDS.

Additional variations on these conditions will be readily apparent to those skilled in the art.

15 Methods for DNA sequencing are well known in the art and may be used to practice any of the embodiments of the invention. The methods may employ such enzymes as the Klenow fragment of DNA polymerase I, SEQUENASE (US Biochemical, Cleveland OH), Taq polymerase (Perkin-Elmer), thermostable T7 polymerase (Amersham Pharmacia Biotech, Piscataway NJ), or combinations of polymerases and proofreading

20 exonucleases such as those found in the ELONGASE amplification system (Life Technologies, Gaithersburg MD). Preferably, sequence preparation is automated with machines such as the Hamilton MICROLAB 2200 (Hamilton, Reno NV), Peltier Thermal Cycler 200 (PTC200; MJ Research, Watertown MA) and the ABI CATALYST 800 (Perkin-Elmer). Sequencing is then carried out using either ABI 373 or 377 DNA

25 sequencing systems (Perkin-Elmer) or the MEGABACE 1000 DNA sequencing system (Molecular Dynamics, Sunnyvale CA). The resulting sequences are analyzed using a variety of algorithms which are well known in the art. (See, e.g., Ausubel, F.M. (1997) Short Protocols in Molecular Biology, John Wiley & Sons, New York NY, unit 7.7; Meyers, R.A. (1995) Molecular Biology and Biotechnology, Wiley VCH, New York NY,

30 pp. 856-853.)

The nucleic acid sequences encoding HSPP may be extended utilizing a partial nucleotide sequence and employing various PCR-based methods known in the art to detect

upstream sequences, such as promoters and regulatory elements. For example, one method which may be employed, restriction-site PCR, uses universal and nested primers to amplify unknown sequence from genomic DNA within a cloning vector. (See, e.g., Sarkar, G. (1993) PCR Methods Applic. 2:318-322.) Another method, inverse PCR, uses 5 primers that extend in divergent directions to amplify unknown sequence from a circularized template. The template is derived from restriction fragments comprising a known genomic locus and surrounding sequences. (See, e.g., Triglia, T. et al. (1988) Nucleic Acids Res. 16:8186.) A third method, capture PCR, involves PCR amplification of DNA fragments adjacent to known sequences in human and yeast artificial chromosome 10 DNA. (See, e.g., Lagerstrom, M. et al. (1991) PCR Methods Applic. 1:111-119.) In this method, multiple restriction enzyme digestions and ligations may be used to insert an engineered double-stranded sequence into a region of unknown sequence before performing PCR. Other methods which may be used to retrieve unknown sequences are known in the art. (See, e.g., Parker, J.D. et al. (1991) Nucleic Acids Res. 19:3055-306). 15 Additionally, one may use PCR, nested primers, and PROMOTERFINDER libraries (Clontech, Palo Alto CA) to walk genomic DNA. This procedure avoids the need to screen libraries and is useful in finding intron/exon junctions. For all PCR-based methods, primers may be designed using commercially available software, such as OLIGO 4.06 20 Primer Analysis software (National Biosciences, Plymouth MN) or another appropriate program, to be about 22 to 30 nucleotides in length, to have a GC content of about 50% or more, and to anneal to the template at temperatures of about 68°C to 72°C.

When screening for full-length cDNAs, it is preferable to use libraries that have been size-selected to include larger cDNAs. In addition, random-primed libraries, which often include sequences containing the 5' regions of genes, are preferable for situations in 25 which an oligo d(T) library does not yield a full-length cDNA. Genomic libraries may be useful for extension of sequence into 5' non-transcribed regulatory regions.

Capillary electrophoresis systems which are commercially available may be used to analyze the size or confirm the nucleotide sequence of sequencing or PCR products. In particular, capillary sequencing may employ flowable polymers for electrophoretic 30 separation, four different nucleotide-specific, laser-stimulated fluorescent dyes, and a charge coupled device camera for detection of the emitted wavelengths. Output/light intensity may be converted to electrical signal using appropriate software (e.g.,

GENOTYPER and SEQUENCE NAVIGATOR, Perkin-Elmer), and the entire process from loading of samples to computer analysis and electronic data display may be computer controlled. Capillary electrophoresis is especially preferable for sequencing small DNA fragments which may be present in limited amounts in a particular sample.

5 In another embodiment of the invention, polynucleotide sequences or fragments thereof which encode HSPP may be cloned in recombinant DNA molecules that direct expression of HSPP, or fragments or functional equivalents thereof, in appropriate host cells. Due to the inherent degeneracy of the genetic code, other DNA sequences which encode substantially the same or a functionally equivalent amino acid sequence may be  
10 produced and used to express HSPP.

The nucleotide sequences of the present invention can be engineered using methods generally known in the art in order to alter HSPP-encoding sequences for a variety of purposes including, but not limited to, modification of the cloning, processing, and/or expression of the gene product. DNA shuffling by random fragmentation and PCR  
15 reassembly of gene fragments and synthetic oligonucleotides may be used to engineer the nucleotide sequences. For example, oligonucleotide-mediated site-directed mutagenesis may be used to introduce mutations that create new restriction sites, alter glycosylation patterns, change codon preference, produce splice variants, and so forth.

In another embodiment, sequences encoding HSPP may be synthesized, in whole  
20 or in part, using chemical methods well known in the art. (See, e.g., Caruthers, M.H. et al. (1980) Nucl. Acids Res. Symp. Ser. 215-223, and Horn, T. et al. (1980) Nucl. Acids Res. Symp. Ser. 225-232.) Alternatively, HSPP itself or a fragment thereof may be synthesized using chemical methods. For example, peptide synthesis can be performed using various solid-phase techniques. (See, e.g., Roberge, J.Y. et al. (1995) Science 269:202-204.)  
25 Automated synthesis may be achieved using the ABI 431A Peptide Synthesizer (Perkin-Elmer). Additionally, the amino acid sequence of HSPP, or any part thereof, may be altered during direct synthesis and/or combined with sequences from other proteins, or any part thereof, to produce a variant polypeptide.

The peptide may be substantially purified by preparative high performance liquid  
30 chromatography. (See, e.g., Chiez, R.M. and F.Z. Regnier (1990) Methods Enzymol. 182:392-421.) The composition of the synthetic peptides may be confirmed by amino acid

analysis or by sequencing. (See, e.g., Creighton, T. (1984) Proteins, Structures and Molecular Properties, WH Freeman, New York NY.)

In order to express a biologically active HSPP, the nucleotide sequences encoding HSPP or derivatives thereof may be inserted into an appropriate expression vector, i.e., a 5 vector which contains the necessary elements for transcriptional and translational control of the inserted coding sequence in a suitable host. These elements include regulatory sequences, such as enhancers, constitutive and inducible promoters, and 5' and 3' untranslated regions in the vector and in polynucleotide sequences encoding HSPP. Such elements may vary in their strength and specificity. Specific initiation signals may also be 10 used to achieve more efficient translation of sequences encoding HSPP. Such signals include the ATG initiation codon and adjacent sequences, e.g. the Kozak sequence. In cases where sequences encoding HSPP and its initiation codon and upstream regulatory sequences are inserted into the appropriate expression vector, no additional transcriptional or translational control signals may be needed. However, in cases where only coding 15 sequence, or a fragment thereof, is inserted, exogenous translational control signals including an in-frame ATG initiation codon should be provided by the vector. Exogenous translational elements and initiation codons may be of various origins, both natural and synthetic. The efficiency of expression may be enhanced by the inclusion of enhancers appropriate for the particular host cell system used. (See, e.g., Scharf, D. et al. (1994) 20 Results Probl. Cell Differ. 20:125-162.)

Methods which are well known to those skilled in the art may be used to construct expression vectors containing sequences encoding HSPP and appropriate transcriptional and translational control elements. These methods include in vitro recombinant DNA techniques, synthetic techniques, and in vivo genetic recombination. (See, e.g., Sambrook, 25 J. et al. (1989) Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Press, Plainview NY, ch. 4, 8, and 16-17; Ausubel, F.M. et al. (1995) Current Protocols in Molecular Biology, John Wiley & Sons, New York NY, ch. 9, 13, and 16.)

A variety of expression vector/host systems may be utilized to contain and express sequences encoding HSPP. These include, but are not limited to, microorganisms such as 30 bacteria transformed with recombinant bacteriophage, plasmid, or cosmid DNA expression vectors; yeast transformed with yeast expression vectors; insect cell systems infected with viral expression vectors (e.g., baculovirus); plant cell systems transformed with viral

expression vectors (e.g., cauliflower mosaic virus, CaMV, or tobacco mosaic virus, TMV) or with bacterial expression vectors (e.g., Ti or pBR322 plasmids); or animal cell systems. The invention is not limited by the host cell employed.

In bacterial systems, a number of cloning and expression vectors may be selected  
5 depending upon the use intended for polynucleotide sequences encoding HSPP. For example, routine cloning, subcloning, and propagation of polynucleotide sequences encoding HSPP can be achieved using a multifunctional E. coli vector such as PBLUESCRIPT (Stratagene, La Jolla CA) or pSPORT1 plasmid (Life Technologies). Ligation of sequences encoding HSPP into the vector's multiple cloning site disrupts the  
10 lacZ gene, allowing a colorimetric screening procedure for identification of transformed bacteria containing recombinant molecules. In addition, these vectors may be useful for in vitro transcription, dideoxy sequencing, single strand rescue with helper phage, and creation of nested deletions in the cloned sequence. (See, e.g., Van Heeke, G. and S.M. Schuster (1989) J. Biol. Chem. 264:5503-5509.) When large quantities of HSPP are  
15 needed, e.g. for the production of antibodies, vectors which direct high level expression of HSPP may be used. For example, vectors containing the strong, inducible T5 or T7 bacteriophage promoter may be used.

Yeast expression systems may be used for production of HSPP. A number of vectors containing constitutive or inducible promoters, such as alpha factor, alcohol  
20 oxidase, and PGH, may be used in the yeast Saccharomyces cerevisiae or Pichia pastoris. In addition, such vectors direct either the secretion or intracellular retention of expressed proteins and enable integration of foreign sequences into the host genome for stable propagation. (See, e.g., Ausubel, 1995, supra; Grant et al. (1987) Methods Enzymol. 153:516-54; and Scorer, C. A. et al. (1994) Bio/Technology 12:181-184.)

25 Plant systems may also be used for expression of HSPP. Transcription of sequences encoding HSPP may be driven viral promoters, e.g., the 35S and 19S promoters of CaMV used alone or in combination with the omega leader sequence from TMV (Takamatsu, N. (1987) EMBO J. 6:307-311). Alternatively, plant promoters such as the small subunit of RUBISCO or heat shock promoters may be used. (See, e.g., Coruzzi, G.  
30 et al. (1984) EMBO J. 3:1671-1680; Broglie, R. et al. (1984) Science 224:838-843; and Winter, J. et al. (1991) Results Probl. Cell Differ. 17:85-105.) These constructs can be introduced into plant cells by direct DNA transformation or pathogen-mediated

transfection. (See, e.g., The McGraw Hill Yearbook of Science and Technology (1992) McGraw Hill, New York NY, pp. 191-196.)

In mammalian cells, a number of viral-based expression systems may be utilized. In cases where an adenovirus is used as an expression vector, sequences encoding HSPP 5 may be ligated into an adenovirus transcription/translation complex consisting of the late promoter and tripartite leader sequence. Insertion in a non-essential E1 or E3 region of the viral genome may be used to obtain infective virus which expresses HSPP in host cells. (See, e.g., Logan, J. and T. Shenk (1984) Proc. Natl. Acad. Sci. 81:3655-3659.) In addition, transcription enhancers, such as the Rous sarcoma virus (RSV) enhancer, may be 10 used to increase expression in mammalian host cells. SV40 or EBV-based vectors may also be used for high-level protein expression.

Human artificial chromosomes (HACs) may also be employed to deliver larger fragments of DNA than can be contained in and expressed from a plasmid. HACs of about 6 kb to 10 Mb are constructed and delivered via conventional delivery methods 15 (liposomes, polycationic amino polymers, or vesicles) for therapeutic purposes. (See, e.g., Harrington, J.J. et al. (1997) Nat Genet. 15:345-355.)

For long term production of recombinant proteins in mammalian systems, stable expression of HSPP in cell lines is preferred. For example, sequences encoding HSPP can be transformed into cell lines using expression vectors which may contain viral origins of 20 replication and/or endogenous expression elements and a selectable marker gene on the same or on a separate vector. Following the introduction of the vector, cells may be allowed to grow for about 1 to 2 days in enriched media before being switched to selective media. The purpose of the selectable marker is to confer resistance to a selective agent, and its presence allows growth and recovery of cells which successfully express the 25 introduced sequences. Resistant clones of stably transformed cells may be propagated using tissue culture techniques appropriate to the cell type.

Any number of selection systems may be used to recover transformed cell lines. These include, but are not limited to, the herpes simplex virus thymidine kinase and adenine phosphoribosyltransferase genes, for use in *tk* or *apr* cells, respectively. (See, 30 e.g., Wigler, M. et al. (1977) Cell 11:223-232; Lowy, I. et al. (1980) Cell 22:817-823.) Also, antimetabolite, antibiotic, or herbicide resistance can be used as the basis for selection. For example, *dhfr* confers resistance to methotrexate; *neo* confers resistance to

the aminoglycosides, neomycin and G-418; and *als* or *pat* confer resistance to chlorsulfuron and phosphinotricin acetyltransferase, respectively. (See, e.g., Wigler, M. et al. (1980) Proc. Natl. Acad. Sci. 77:3567-3570; Colbere-Garapin, F. et al. (1981) J. Mol. Biol. 150:1-14.) Additional selectable genes have been described, e.g., *trpB* and *hisD*, 5 which alter cellular requirements for metabolites. (See, e.g., Hartman, S.C. and R.C. Mulligan (1988) Proc. Natl. Acad. Sci. 85:8047-8051.) Visible markers, e.g., anthocyanins, green fluorescent proteins (GFP; Clontech),  $\beta$  glucuronidase and its substrate  $\beta$ -glucuronide, or luciferase and its substrate luciferin may be used. These markers can be used not only to identify transformants, but also to quantify the amount of 10 transient or stable protein expression attributable to a specific vector system. (See, e.g., Rhodes, C.A. (1995) Methods Mol. Biol. 55:121-131.)

Although the presence/absence of marker gene expression suggests that the gene of interest is also present, the presence and expression of the gene may need to be confirmed. For example, if the sequence encoding HSPP is inserted within a marker gene sequence, 15 transformed cells containing sequences encoding HSPP can be identified by the absence of marker gene function. Alternatively, a marker gene can be placed in tandem with a sequence encoding HSPP under the control of a single promoter. Expression of the marker gene in response to induction or selection usually indicates expression of the tandem gene as well.

20 In general, host cells that contain the nucleic acid sequence encoding HSPP and that express HSPP may be identified by a variety of procedures known to those of skill in the art. These procedures include, but are not limited to, DNA-DNA or DNA-RNA hybridizations, PCR amplification, and protein bioassay or immunoassay techniques which include membrane, solution, or chip based technologies for the detection and/or 25 quantification of nucleic acid or protein sequences.

Immunological methods for detecting and measuring the expression of HSPP using either specific polyclonal or monoclonal antibodies are known in the art. Examples of such techniques include enzyme-linked immunosorbent assays (ELISAs), radioimmunoassays (RIAs), and fluorescence activated cell sorting (FACS). A two-site, 30 monoclonal-based immunoassay utilizing monoclonal antibodies reactive to two non-interfering epitopes on HSPP is preferred, but a competitive binding assay may be employed. These and other assays are well known in the art. (See, e.g., Hampton, R. et al.

(1990) Serological Methods, a Laboratory Manual, APS Press, St Paul MN, Sect. IV; Coligan, J. E. et al. (1997) Current Protocols in Immunology, Greene Pub. Associates and Wiley-Interscience, New York NY; and Pound, J.D. (1998) Immunochemical Protocols, Humana Press, Totowa NJ).

5 A wide variety of labels and conjugation techniques are known by those skilled in the art and may be used in various nucleic acid and amino acid assays. Means for producing labeled hybridization or PCR probes for detecting sequences related to polynucleotides encoding HSPP include oligolabeling, nick translation, end-labeling, or PCR amplification using a labeled nucleotide. Alternatively, the sequences encoding 10 HSPP, or any fragments thereof, may be cloned into a vector for the production of an mRNA probe. Such vectors are known in the art, are commercially available, and may be used to synthesize RNA probes in vitro by addition of an appropriate RNA polymerase such as T7, T3, or SP6 and labeled nucleotides. These procedures may be conducted using a variety of commercially available kits, such as those provided by Amersham Pharmacia 15 Biotech, Promega (Madison WI), and US Biochemical. Suitable reporter molecules or labels which may be used for ease of detection include radionuclides, enzymes, fluorescent, chemiluminescent, or chromogenic agents, as well as substrates, cofactors, inhibitors, magnetic particles, and the like.

Host cells transformed with nucleotide sequences encoding HSPP may be cultured 20 under conditions suitable for the expression and recovery of the protein from cell culture. The protein produced by a transformed cell may be secreted or retained intracellularly depending on the sequence and/or the vector used. As will be understood by those of skill in the art, expression vectors containing polynucleotides which encode HSPP may be designed to contain signal sequences which direct secretion of HSPP through a prokaryotic 25 or eukaryotic cell membrane.

In addition, a host cell strain may be chosen for its ability to modulate expression of the inserted sequences or to process the expressed protein in the desired fashion. Such modifications of the polypeptide include, but are not limited to, acetylation, carboxylation, glycosylation, phosphorylation, lipidation, and acylation. Post-translational processing 30 which cleaves a "prepro" form of the protein may also be used to specify protein targeting, folding, and/or activity. Different host cells which have specific cellular machinery and characteristic mechanisms for post-translational activities (e.g., CHO, HeLa, MDCK,

HEK293, and WI38), are available from the American Type Culture Collection (ATCC, Manassas, VA) and may be chosen to ensure the correct modification and processing of the foreign protein.

In another embodiment of the invention, natural, modified, or recombinant nucleic acid sequences encoding HSPP may be ligated to a heterologous sequence resulting in translation of a fusion protein in any of the aforementioned host systems. For example, a chimeric HSPP protein containing a heterologous moiety that can be recognized by a commercially available antibody may facilitate the screening of peptide libraries for inhibitors of HSPP activity. Heterologous protein and peptide moieties may also facilitate purification of fusion proteins using commercially available affinity matrices. Such moieties include, but are not limited to, glutathione S-transferase (GST), maltose binding protein (MBP), thioredoxin (Trx), calmodulin binding peptide (CBP), 6-His, FLAG, *c-myc*, and hemagglutinin (HA). GST, MBP, Trx, CBP, and 6-His enable purification of their cognate fusion proteins on immobilized glutathione, maltose, phenylarsine oxide, calmodulin, and metal-chelate resins, respectively. FLAG, *c-myc*, and hemagglutinin (HA) enable immunoaffinity purification of fusion proteins using commercially available monoclonal and polyclonal antibodies that specifically recognize these epitope tags. A fusion protein may also be engineered to contain a proteolytic cleavage site located between the HSPP encoding sequence and the heterologous protein sequence, so that HSPP may be cleaved away from the heterologous moiety following purification. Methods for fusion protein expression and purification are discussed in Ausubel (1995, supra, ch 10). A variety of commercially available kits may also be used to facilitate expression and purification of fusion proteins.

In a further embodiment of the invention, synthesis of radiolabeled HSPP may be achieved in vitro using the TNT rabbit reticulocyte lysate or wheat germ extract systems (Promega). These systems couple transcription and translation of protein-coding sequences operably associated with the T7, T3, or SP6 promoters. Translation takes place in the presence of a radiolabeled amino acid precursor, preferably <sup>35</sup>S-methionine.

Fragments of HSPP may be produced not only by recombinant production, but also by direct peptide synthesis using solid-phase techniques. (See, e.g., Creighton, supra, pp. 55-60.) Protein synthesis may be performed by manual techniques or by automation. Automated synthesis may be achieved, for example, using the ABI 431A Peptide

Synthesizer (Perkin-Elmer). Various fragments of HSPP may be synthesized separately and then combined to produce the full length molecule.

## THERAPEUTICS

5       Chemical and structural similarity, e.g., in the context of sequences and motifs, exists between regions of HSPP and signal peptide sequences. In addition, chemical and structural similarity, in the context of sequences and motifs, exists between HSPP-66 and prostatic steroid-binding C3 precursor from rat (GI 206453); between HSPP-68 and TWIK-related acid-sensitive K<sup>+</sup>channel from human (GI 2465542); and between HSPP-92  
10      and tyrosine specific protein phosphatases (PROSITE PDOC00323). In addition, the expression of HSPP is closely associated with proliferative, cancerous, inflamed, cardiovascular, nervous, reproductive,  
hematopoietic/immune, and developmental tissue. Therefore, HSPP appears to play a role in cell proliferative disorders including cancer; inflammation; and cardiovascular,  
15      neurological, reproductive, and developmental disorders. In the treatment of cell proliferative disorders including cancer; inflammation; and cardiovascular, neurological, reproductive, and developmental disorders associated with increased HSPP expression or activity, it is desirable to decrease the expression or activity of HSPP. In the treatment of the above conditions associated with decreased HSPP expression or activity, it is desirable  
20      to increase the expression or activity of HSPP.

Therefore, in one embodiment, HSPP or a fragment or derivative thereof may be administered to a subject to treat or prevent a disorder associated with decreased expression or activity of HSPP. Examples of such disorders include, but are not limited to, cell proliferative disorders such as actinic keratosis, arteriosclerosis, atherosclerosis, bursitis, cirrhosis, hepatitis, mixed connective tissue disease (MCTD), myelofibrosis, paroxysmal nocturnal hemoglobinuria, polycythemia vera, psoriasis, primary thrombocythemia, and cancers including adenocarcinoma, leukemia, lymphoma, melanoma, myeloma, sarcoma, teratocarcinoma, and, in particular, cancers of the adrenal gland, bladder, bone, bone marrow, brain, breast, cervix, gall bladder, ganglia, 25      gastrointestinal tract, heart, kidney, liver, lung, muscle, ovary, pancreas, parathyroid, penis, prostate, salivary glands, skin, spleen, testis, thymus, thyroid, and uterus; inflammatory disorders, such as acquired immunodeficiency syndrome (AIDS), Addison's  
30

disease, adult respiratory distress syndrome, allergies, ankylosing spondylitis, amyloidosis, anemia, asthma, atherosclerosis, autoimmune hemolytic anemia, autoimmune thyroiditis, autoimmune polyenocrinopathy-candidiasis-ectodermal dystrophy (APECED), bronchitis, cholecystitis, contact dermatitis, Crohn's disease, atopic dermatitis,

5 dermatomyositis, diabetes mellitus, emphysema, episodic lymphopenia with lymphocytotoxins, erythroblastosis fetalis, erythema nodosum, atrophic gastritis, glomerulonephritis, Goodpasture's syndrome, gout, Graves' disease, Hashimoto's thyroiditis, hypereosinophilia, irritable bowel syndrome, multiple sclerosis, myasthenia gravis, myocardial or pericardial inflammation, osteoarthritis, osteoporosis, pancreatitis,

10 polymyositis, psoriasis, Reiter's syndrome, rheumatoid arthritis, scleroderma, Sjögren's syndrome, systemic anaphylaxis, systemic lupus erythematosus, systemic sclerosis, thrombocytopenic purpura, ulcerative colitis, uveitis, Werner syndrome, complications of cancer, hemodialysis, and extracorporeal circulation, viral, bacterial, fungal, parasitic, protozoal, and helminthic infections, and trauma; cardiovascular disorders including

15 disorders of the blood vessels such as arteriovenous fistula, atherosclerosis, hypertension, vasculitis, Raynaud's disease, aneurysms, arterial dissections, varicose veins, thrombophlebitis and phlebothrombosis, and vascular tumors; disorders of the heart such as congestive heart failure, ischemic heart disease, angina pectoris, myocardial infarction, hypertensive heart disease, degenerative valvular heart disease, calcific aortic valve

20 stenosis, congenitally bicuspid aortic valve, mitral annular calcification, mitral valve prolapse, rheumatic fever and rheumatic heart disease, infective endocarditis, nonbacterial thrombotic endocarditis, endocarditis of systemic lupus erythematosus, carcinoid heart disease, cardiomyopathy, myocarditis, pericarditis, neoplastic heart disease, and congenital heart disease; and disorders of the lungs such as congenital lung anomalies, atelectasis,

25 pulmonary congestion and edema, pulmonary embolism, pulmonary hemorrhage, pulmonary infarction, pulmonary hypertension, vascular sclerosis, obstructive pulmonary disease, restrictive pulmonary disease, chronic obstructive pulmonary disease, emphysema, chronic bronchitis, bronchial asthma, bronchiectasis, bacterial pneumonia, viral and mycoplasmal pneumonia, lung abscess, pulmonary tuberculosis, diffuse

30 interstitial diseases, pneumoconioses, sarcoidosis, idiopathic pulmonary fibrosis, desquamative interstitial pneumonitis, hypersensitivity pneumonitis, pulmonary eosinophilia bronchiolitis obliterans-organizing pneumonia, diffuse pulmonary

hemorrhage syndromes, Goodpasture's syndromes, idiopathic pulmonary hemosiderosis, pulmonary involvement in collagen-vascular disorders, pulmonary alveolar proteinosis, lung tumors, inflammatory and noninflammatory pleural effusions, pneumothorax, and pleural tumors; neurological disorders such as epilepsy, ischemic cerebrovascular disease, 5 stroke, cerebral neoplasms, Alzheimer's disease, Pick's disease, Huntington's disease, dementia, Parkinson's disease and other extrapyramidal disorders, amyotrophic lateral sclerosis and other motor neuron disorders, progressive neural muscular atrophy, retinitis pigmentosa, hereditary ataxias, multiple sclerosis and other demyelinating diseases, bacterial and viral meningitis, brain abscess, subdural empyema, epidural abscess, 10 suppurative intracranial thrombophlebitis, myelitis and radiculitis, viral central nervous system disease; prion diseases including kuru, Creutzfeldt-Jakob disease, and Gerstmann-Straussler-Scheinker syndrome; fatal familial insomnia, nutritional and metabolic diseases of the nervous system, neurofibromatosis, tuberous sclerosis, cerebelloretinal hemangioblastomatosis, encephalotrigeminal syndrome, mental retardation and other 15 developmental disorders of the central nervous system, cerebral palsy, neuroskeletal disorders, autonomic nervous system disorders, cranial nerve disorders, spinal cord diseases, muscular dystrophy and other neuromuscular disorders, peripheral nervous system disorders, dermatomyositis and polymyositis; inherited, metabolic, endocrine, and toxic myopathies; myasthenia gravis, periodic paralysis; mental disorders including mood, 20 anxiety, and schizophrenic disorders; akathesia, amnesia, catatonia, diabetic neuropathy, tardive dyskinesia, dystonias, paranoid psychoses, postherpetic neuralgia, and Tourette's disorder; reproductive disorders such as disorders of prolactin production; infertility, including tubal disease, ovulatory defects, and endometriosis; disruptions of the estrous cycle, disruptions of the menstrual cycle, polycystic ovary syndrome, ovarian 25 hyperstimulation syndrome, endometrial and ovarian tumors, uterine fibroids, autoimmune disorders, ectopic pregnancies, and teratogenesis; cancer of the breast, fibrocystic breast disease, and galactorrhea; disruptions of spermatogenesis, abnormal sperm physiology, cancer of the testis, cancer of the prostate, benign prostatic hyperplasia, prostatitis, Peyronie's disease, carcinoma of the male breast, and gynecomastia; and developmental 30 disorders, such as renal tubular acidosis, anemia, Cushing's syndrome, achondroplastic dwarfism, Duchenne and Becker muscular dystrophy, epilepsy, gonadal dysgenesis, WAGR syndrome (Wilms' tumor, aniridia, genitourinary abnormalities, and mental

retardation), Smith-Magenis syndrome, myelodysplastic syndrome, hereditary mucoepithelial dysplasia, hereditary keratodermas, hereditary neuropathies such as Charcot-Marie-Tooth disease and neurofibromatosis, hypothyroidism, hydrocephalus, seizure disorders such as Sydenham's chorea and cerebral palsy, spina bifida, 5 anencephaly, craniorachischisis, congenital glaucoma, cataract, and sensorineural hearing loss.

In another embodiment, a vector capable of expressing HSPP or a fragment or derivative thereof may be administered to a subject to treat or prevent a disorder associated with decreased expression or activity of HSPP including, but not limited to, those 10 described above.

In a further embodiment, a pharmaceutical composition comprising a substantially purified HSPP in conjunction with a suitable pharmaceutical carrier may be administered to a subject to treat or prevent a disorder associated with decreased expression or activity of HSPP including, but not limited to, those provided above.

15 In still another embodiment, an agonist which modulates the activity of HSPP may be administered to a subject to treat or prevent a disorder associated with decreased expression or activity of HSPP including, but not limited to, those listed above.

In a further embodiment, an antagonist of HSPP may be administered to a subject to treat or prevent a disorder associated with increased expression or activity of HSPP.

20 Examples of such disorders include, but are not limited to, those described above. In one aspect, an antibody which specifically binds HSPP may be used directly as an antagonist or indirectly as a targeting or delivery mechanism for bringing a pharmaceutical agent to cells or tissue which express HSPP.

In an additional embodiment, a vector expressing the complement of the 25 polynucleotide encoding HSPP may be administered to a subject to treat or prevent a disorder associated with increased expression or activity of HSPP including, but not limited to, those described above.

In other embodiments, any of the proteins, antagonists, antibodies, agonists, complementary sequences, or vectors of the invention may be administered in combination 30 with other appropriate therapeutic agents. Selection of the appropriate agents for use in combination therapy may be made by one of ordinary skill in the art, according to conventional pharmaceutical principles. The combination of therapeutic agents may act

synergistically to effect the treatment or prevention of the various disorders described above. Using this approach, one may be able to achieve therapeutic efficacy with lower dosages of each agent, thus reducing the potential for adverse side effects.

An antagonist of HSPP may be produced using methods which are generally known in the art. In particular, purified HSPP may be used to produce antibodies or to screen libraries of pharmaceutical agents to identify those which specifically bind HSPP. Antibodies to HSPP may also be generated using methods that are well known in the art. Such antibodies may include, but are not limited to, polyclonal, monoclonal, chimeric, and single chain antibodies, Fab fragments, and fragments produced by a Fab expression library. Neutralizing antibodies (i.e., those which inhibit dimer formation) are especially preferred for therapeutic use.

For the production of antibodies, various hosts including goats, rabbits, rats, mice, humans, and others may be immunized by injection with HSPP or with any fragment or oligopeptide thereof which has immunogenic properties. Depending on the host species, various adjuvants may be used to increase immunological response. Such adjuvants include, but are not limited to, Freund's, mineral gels such as aluminum hydroxide, and surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, KLH, and dinitrophenol. Among adjuvants used in humans, BCG (bacilli Calmette-Guerin) and Corynebacterium parvum are especially preferable.

It is preferred that the oligopeptides, peptides, or fragments used to induce antibodies to HSPP have an amino acid sequence consisting of at least about 5 amino acids, and, more preferably, of at least about 10 amino acids. It is also preferable that these oligopeptides, peptides, or fragments are identical to a portion of the amino acid sequence of the natural protein and contain the entire amino acid sequence of a small, naturally occurring molecule. Short stretches of HSPP amino acids may be fused with those of another protein, such as KLH, and antibodies to the chimeric molecule may be produced.

Monoclonal antibodies to HSPP may be prepared using any technique which provides for the production of antibody molecules by continuous cell lines in culture. These include, but are not limited to, the hybridoma technique, the human B-cell hybridoma technique, and the EBV-hybridoma technique. (See, e.g., Kohler, G. et al. (1975) *Nature* 256:495-497; Kozbor, D. et al. (1985) *J. Immunol. Methods* 81:31-42;

Cote, R.J. et al. (1983) Proc. Natl. Acad. Sci. 80:2026-2030; and Cole, S.P. et al. (1984) Mol. Cell Biol. 62:109-120.)

In addition, techniques developed for the production of "chimeric antibodies," such as the splicing of mouse antibody genes to human antibody genes to obtain a molecule 5 with appropriate antigen specificity and biological activity, can be used. (See, e.g., Morrison, S.L. et al. (1984) Proc. Natl. Acad. Sci. 81:6851-6855; Neuberger, M.S. et al. (1984) Nature 312:604-608; and Takeda, S. et al. (1985) Nature 314:452-454.) Alternatively, techniques described for the production of single chain antibodies may be adapted, using methods known in the art, to produce HSPP-specific single chain 10 antibodies. Antibodies with related specificity, but of distinct idiotypic composition, may be generated by chain shuffling from random combinatorial immunoglobulin libraries. (See, e.g., Burton D.R. (1991) Proc. Natl. Acad. Sci. 88:10134-10137.)

Antibodies may also be produced by inducing in vivo production in the lymphocyte population or by screening immunoglobulin libraries or panels of highly 15 specific binding reagents as disclosed in the literature. (See, e.g., Orlandi, R. et al. (1989) Proc. Natl. Acad. Sci. 86: 3833-3837; Winter, G. et al. (1991) Nature 349:293-299.)

Antibody fragments which contain specific binding sites for HSPP may also be generated. For example, such fragments include, but are not limited to, F(ab')2 fragments produced by pepsin digestion of the antibody molecule and Fab fragments generated by 20 reducing the disulfide bridges of the F(ab')2 fragments. Alternatively, Fab expression libraries may be constructed to allow rapid and easy identification of monoclonal Fab fragments with the desired specificity. (See, e.g., Huse, W.D. et al. (1989) Science 246:1275-1281.)

Various immunoassays may be used for screening to identify antibodies having the 25 desired specificity. Numerous protocols for competitive binding or immunoradiometric assays using either polyclonal or monoclonal antibodies with established specificities are well known in the art. Such immunoassays typically involve the measurement of complex formation between HSPP and its specific antibody. A two-site, monoclonal-based immunoassay utilizing monoclonal antibodies reactive to two non-interfering HSPP 30 epitopes is preferred, but a competitive binding assay may also be employed (Pound, supra).

Various methods such as Scatchard analysis in conjunction with radioimmunoassay techniques may be used to assess the affinity of antibodies for HSPP. Affinity is expressed as an association constant,  $K_a$ , which is defined as the molar concentration of HSPP-antibody complex divided by the molar concentrations of free antigen and free antibody under equilibrium conditions. The  $K_a$  determined for a preparation of polyclonal antibodies, which are heterogeneous in their affinities for multiple HSPP epitopes, represents the average affinity, or avidity, of the antibodies for HSPP. The  $K_a$  determined for a preparation of monoclonal antibodies, which are monospecific for a particular HSPP epitope, represents a true measure of affinity. High-affinity antibody preparations with  $K_a$  ranging from about  $10^9$  to  $10^{12}$  L/mole are preferred for use in immunoassays in which the HSPP-antibody complex must withstand rigorous manipulations. Low-affinity antibody preparations with  $K_a$  ranging from about  $10^6$  to  $10^7$  L/mole are preferred for use in immunopurification and similar procedures which ultimately require dissociation of HSPP, preferably in active form, from the antibody

15 (Catty, D. (1988) Antibodies, Volume I: A Practical Approach, IRL Press, Washington, DC; Liddell, J. E. and Cryer, A. (1991) A Practical Guide to Monoclonal Antibodies, John Wiley & Sons, New York NY).

The titer and avidity of polyclonal antibody preparations may be further evaluated to determine the quality and suitability of such preparations for certain downstream applications. For example, a polyclonal antibody preparation containing at least 1-2 mg specific antibody/ml, preferably 5-10 mg specific antibody/ml, is preferred for use in procedures requiring precipitation of HSPP-antibody complexes. Procedures for evaluating antibody specificity, titer, and avidity, and guidelines for antibody quality and usage in various applications, are generally available. (See, e.g., Catty, supra, and Coligan

25 et al. supra.)

In another embodiment of the invention, the polynucleotides encoding HSPP, or any fragment or complement thereof, may be used for therapeutic purposes. In one aspect, the complement of the polynucleotide encoding HSPP may be used in situations in which it would be desirable to block the transcription of the mRNA. In particular, cells may be transformed with sequences complementary to polynucleotides encoding HSPP. Thus, complementary molecules or fragments may be used to modulate HSPP activity, or to achieve regulation of gene function. Such technology is now well known in the art, and

sense or antisense oligonucleotides or larger fragments can be designed from various locations along the coding or control regions of sequences encoding HSPP.

Expression vectors derived from retroviruses, adenoviruses, or herpes or vaccinia viruses, or from various bacterial plasmids, may be used for delivery of nucleotide sequences to the targeted organ, tissue, or cell population. Methods which are well known to those skilled in the art can be used to construct vectors to express nucleic acid sequences complementary to the polynucleotides encoding HSPP. (See, e.g., Sambrook, supra; Ausubel, 1995, supra.)

Genes encoding HSPP can be turned off by transforming a cell or tissue with expression vectors which express high levels of a polynucleotide, or fragment thereof, encoding HSPP. Such constructs may be used to introduce untranslatable sense or antisense sequences into a cell. Even in the absence of integration into the DNA, such vectors may continue to transcribe RNA molecules until they are disabled by endogenous nucleases. Transient expression may last for a month or more with a non-replicating vector, and may last even longer if appropriate replication elements are part of the vector system.

As mentioned above, modifications of gene expression can be obtained by designing complementary sequences or antisense molecules (DNA, RNA, or PNA) to the control, 5', or regulatory regions of the gene encoding HSPP. Oligonucleotides derived from the transcription initiation site, e.g., between about positions -10 and +10 from the start site, are preferred. Similarly, inhibition can be achieved using triple helix base-pairing methodology. Triple helix pairing is useful because it causes inhibition of the ability of the double helix to open sufficiently for the binding of polymerases, transcription factors, or regulatory molecules. Recent therapeutic advances using triplex DNA have been described in the literature. (See, e.g., Gee, J.E. et al. (1994) in Huber, B.E. and B.I. Carr, Molecular and Immunologic Approaches, Futura Publishing, Mt. Kisco NY, pp. 163-177.) A complementary sequence or antisense molecule may also be designed to block translation of mRNA by preventing the transcript from binding to ribosomes.

Ribozymes, enzymatic RNA molecules, may also be used to catalyze the specific cleavage of RNA. The mechanism of ribozyme action involves sequence-specific hybridization of the ribozyme molecule to complementary target RNA, followed by

endonucleolytic cleavage. For example, engineered hammerhead motif ribozyme molecules may specifically and efficiently catalyze endonucleolytic cleavage of sequences encoding HSPP.

Specific ribozyme cleavage sites within any potential RNA target are initially identified by scanning the target molecule for ribozyme cleavage sites, including the following sequences: GUA, GUU, and GUC. Once identified, short RNA sequences of between 15 and 20 ribonucleotides, corresponding to the region of the target gene containing the cleavage site, may be evaluated for secondary structural features which may render the oligonucleotide inoperable. The suitability of candidate targets may also be evaluated by testing accessibility to hybridization with complementary oligonucleotides using ribonuclease protection assays.

Complementary ribonucleic acid molecules and ribozymes of the invention may be prepared by any method known in the art for the synthesis of nucleic acid molecules.

These include techniques for chemically synthesizing oligonucleotides such as solid phase phosphoramidite chemical synthesis. Alternatively, RNA molecules may be generated by in vitro and in vivo transcription of DNA sequences encoding HSPP. Such DNA sequences may be incorporated into a wide variety of vectors with suitable RNA polymerase promoters such as T7 or SP6. Alternatively, these cDNA constructs that synthesize complementary RNA, constitutively or inducibly, can be introduced into cell lines, cells, or tissues.

RNA molecules may be modified to increase intracellular stability and half-life. Possible modifications include, but are not limited to, the addition of flanking sequences at the 5' and/or 3' ends of the molecule, or the use of phosphorothioate or 2' O-methyl rather than phosphodiesterase linkages within the backbone of the molecule. This concept is inherent in the production of PNAs and can be extended in all of these molecules by the inclusion of nontraditional bases such as inosine, queosine, and wybutosine, as well as acetyl-, methyl-, thio-, and similarly modified forms of adenine, cytidine, guanine, thymine, and uridine which are not as easily recognized by endogenous endonucleases.

Many methods for introducing vectors into cells or tissues are available and equally suitable for use in vivo, in vitro, and ex vivo. For ex vivo therapy, vectors may be introduced into stem cells taken from the patient and clonally propagated for autologous transplant back into that same patient. Delivery by transfection, by liposome injections, or

by polycationic amino polymers may be achieved using methods which are well known in the art. (See, e.g., Goldman, C.K. et al. (1997) *Nature Biotechnology* 15:462-466.)

Any of the therapeutic methods described above may be applied to any subject in need of such therapy, including, for example, mammals such as dogs, cats, cows, horses, 5 rabbits, monkeys, and most preferably, humans.

An additional embodiment of the invention relates to the administration of a pharmaceutical or sterile composition, in conjunction with a pharmaceutically acceptable carrier, for any of the therapeutic effects discussed above. Such pharmaceutical compositions may consist of HSPP, antibodies to HSPP, and mimetics, agonists, 10 antagonists, or inhibitors of HSPP. The compositions may be administered alone or in combination with at least one other agent, such as a stabilizing compound, which may be administered in any sterile, biocompatible pharmaceutical carrier including, but not limited to, saline, buffered saline, dextrose, and water. The compositions may be administered to a patient alone, or in combination with other agents, drugs, or hormones.

15 The pharmaceutical compositions utilized in this invention may be administered by any number of routes including, but not limited to, oral, intravenous, intramuscular, intra-arterial, intramedullary, intrathecal, intraventricular, transdermal, subcutaneous, intraperitoneal, intranasal, enteral, topical, sublingual, or rectal means.

In addition to the active ingredients, these pharmaceutical compositions may 20 contain suitable pharmaceutically-acceptable carriers comprising excipients and auxiliaries which facilitate processing of the active compounds into preparations which can be used pharmaceutically. Further details on techniques for formulation and administration may be found in the latest edition of Remington's Pharmaceutical Sciences (Maack Publishing, Easton PA).

25 Pharmaceutical compositions for oral administration can be formulated using pharmaceutically acceptable carriers well known in the art in dosages suitable for oral administration. Such carriers enable the pharmaceutical compositions to be formulated as tablets, pills, dragees, capsules, liquids, gels, syrups, slurries, suspensions, and the like, for ingestion by the patient.

30 Pharmaceutical preparations for oral use can be obtained through combining active compounds with solid excipient and processing the resultant mixture of granules (optionally, after grinding) to obtain tablets or dragee cores. Suitable auxiliaries can be

added, if desired. Suitable excipients include carbohydrate or protein fillers, such as sugars, including lactose, sucrose, mannitol, and sorbitol; starch from corn, wheat, rice, potato, or other plants; cellulose, such as methyl cellulose, hydroxypropylmethyl-cellulose, or sodium carboxymethylcellulose; gums, including 5 arabic and tragacanth; and proteins, such as gelatin and collagen. If desired, disintegrating or solubilizing agents may be added, such as the cross-linked polyvinyl pyrrolidone, agar, and alginic acid or a salt thereof, such as sodium alginate.

10 Dragee cores may be used in conjunction with suitable coatings, such as concentrated sugar solutions, which may also contain gum arabic, talc, polyvinylpyrrolidone, carbopol gel, polyethylene glycol, and/or titanium dioxide, lacquer solutions, and suitable organic solvents or solvent mixtures. Dyestuffs or pigments may be added to the tablets or dragee coatings for product identification or to characterize the quantity of active compound, i.e., dosage.

15 Pharmaceutical preparations which can be used orally include push-fit capsules made of gelatin, as well as soft, sealed capsules made of gelatin and a coating, such as glycerol or sorbitol. Push-fit capsules can contain active ingredients mixed with fillers or binders, such as lactose or starches, lubricants, such as talc or magnesium stearate, and, optionally, stabilizers. In soft capsules, the active compounds may be dissolved or suspended in suitable liquids, such as fatty oils, liquid, or liquid polyethylene glycol with 20 or without stabilizers.

25 Pharmaceutical formulations suitable for parenteral administration may be formulated in aqueous solutions, preferably in physiologically compatible buffers such as Hanks' solution, Ringer's solution, or physiologically buffered saline. Aqueous injection suspensions may contain substances which increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol, or dextran. Additionally, suspensions of the active compounds may be prepared as appropriate oily injection suspensions. Suitable lipophilic solvents or vehicles include fatty oils, such as sesame oil, or synthetic fatty acid esters, such as ethyl oleate, triglycerides, or liposomes. Non-lipid polycationic amino polymers may also be used for delivery. Optionally, the suspension may also contain 30 suitable stabilizers or agents to increase the solubility of the compounds and allow for the preparation of highly concentrated solutions.

For topical or nasal administration, penetrants appropriate to the particular barrier to be permeated are used in the formulation. Such penetrants are generally known in the art.

The pharmaceutical compositions of the present invention may be manufactured in 5 a manner that is known in the art, e.g., by means of conventional mixing, dissolving, granulating, dragee-making, levigating, emulsifying, encapsulating, entrapping, or lyophilizing processes.

The pharmaceutical composition may be provided as a salt and can be formed with many acids, including but not limited to, hydrochloric, sulfuric, acetic, lactic, tartaric, 10 malic, and succinic acid. Salts tend to be more soluble in aqueous or other protonic solvents than are the corresponding free base forms. In other cases, the preferred preparation may be a lyophilized powder which may contain any or all of the following: 1 mM to 50 mM histidine, 0.1% to 2% sucrose, and 2% to 7% mannitol, at a pH range of 4.5 to 5.5, that is combined with buffer prior to use.

15 After pharmaceutical compositions have been prepared, they can be placed in an appropriate container and labeled for treatment of an indicated condition. For administration of HSPP, such labeling would include amount, frequency, and method of administration.

Pharmaceutical compositions suitable for use in the invention include compositions 20 wherein the active ingredients are contained in an effective amount to achieve the intended purpose. The determination of an effective dose is well within the capability of those skilled in the art.

For any compound, the therapeutically effective dose can be estimated initially either in cell culture assays, e.g., of neoplastic cells or in animal models such as mice, rats, 25 rabbits, dogs, or pigs. An animal model may also be used to determine the appropriate concentration range and route of administration. Such information can then be used to determine useful doses and routes for administration in humans.

A therapeutically effective dose refers to that amount of active ingredient, for example HSPP or fragments thereof, antibodies of HSPP, and agonists, antagonists or 30 inhibitors of HSPP, which ameliorates the symptoms or condition. Therapeutic efficacy and toxicity may be determined by standard pharmaceutical procedures in cell cultures or with experimental animals, such as by calculating the ED<sub>50</sub> (the dose therapeutically

effective in 50% of the population) or LD<sub>50</sub> (the dose lethal to 50% of the population) statistics. The dose ratio of toxic to therapeutic effects is the therapeutic index, and it can be expressed as the LD<sub>50</sub>/ED<sub>50</sub> ratio. Pharmaceutical compositions which exhibit large therapeutic indices are preferred. The data obtained from cell culture assays and animal studies are used to formulate a range of dosage for human use. The dosage contained in such compositions is preferably within a range of circulating concentrations that includes the ED<sub>50</sub> with little or no toxicity. The dosage varies within this range depending upon the dosage form employed, the sensitivity of the patient, and the route of administration.

The exact dosage will be determined by the practitioner, in light of factors related to the subject requiring treatment. Dosage and administration are adjusted to provide sufficient levels of the active moiety or to maintain the desired effect. Factors which may be taken into account include the severity of the disease state, the general health of the subject, the age, weight, and gender of the subject, time and frequency of administration, drug combination(s), reaction sensitivities, and response to therapy. Long-acting pharmaceutical compositions may be administered every 3 to 4 days, every week, or biweekly depending on the half-life and clearance rate of the particular formulation.

Normal dosage amounts may vary from about 0.1  $\mu$ g to 100,000  $\mu$ g, up to a total dose of about 1 gram, depending upon the route of administration. Guidance as to particular dosages and methods of delivery is provided in the literature and generally available to practitioners in the art. Those skilled in the art will employ different formulations for nucleotides than for proteins or their inhibitors. Similarly, delivery of polynucleotides or polypeptides will be specific to particular cells, conditions, locations, etc.

## 25 DIAGNOSTICS

In another embodiment, antibodies which specifically bind HSPP may be used for the diagnosis of disorders characterized by expression of HSPP, or in assays to monitor patients being treated with HSPP or agonists, antagonists, or inhibitors of HSPP.

Antibodies useful for diagnostic purposes may be prepared in the same manner as described above for therapeutics. Diagnostic assays for HSPP include methods which utilize the antibody and a label to detect HSPP in human body fluids or in extracts of cells or tissues. The antibodies may be used with or without modification, and may be labeled

by covalent or non-covalent attachment of a reporter molecule. A wide variety of reporter molecules, several of which are described above, are known in the art and may be used.

A variety of protocols for measuring HSPP, including ELISAs, RIAs, and FACS, are known in the art and provide a basis for diagnosing altered or abnormal levels of HSPP expression. Normal or standard values for HSPP expression are established by combining body fluids or cell extracts taken from normal mammalian subjects, preferably human, with antibody to HSPP under conditions suitable for complex formation. The amount of standard complex formation may be quantitated by various methods, preferably by photometric means. Quantities of HSPP expressed in subject, control, and disease samples from biopsied tissues are compared with the standard values. Deviation between standard and subject values establishes the parameters for diagnosing disease.

In another embodiment of the invention, the polynucleotides encoding HSPP may be used for diagnostic purposes. The polynucleotides which may be used include oligonucleotide sequences, complementary RNA and DNA molecules, and PNAs. The polynucleotides may be used to detect and quantitate gene expression in biopsied tissues in which expression of HSPP may be correlated with disease. The diagnostic assay may be used to determine absence, presence, and excess expression of HSPP, and to monitor regulation of HSPP levels during therapeutic intervention.

In one aspect, hybridization with PCR probes which are capable of detecting polynucleotide sequences, including genomic sequences, encoding HSPP or closely related molecules may be used to identify nucleic acid sequences which encode HSPP. The specificity of the probe, whether it is made from a highly specific region, e.g., the 5' regulatory region, or from a less specific region, e.g., a conserved motif, and the stringency of the hybridization or amplification (maximal, high, intermediate, or low), will determine whether the probe identifies only naturally occurring sequences encoding HSPP, allelic variants, or related sequences.

Probes may also be used for the detection of related sequences, and should preferably have at least 50% sequence identity to any of the HSPP encoding sequences. The hybridization probes of the subject invention may be DNA or RNA and may be derived from the sequence of SEQ ID NO:135-268 or from genomic sequences including promoters, enhancers, and introns of the HSPP gene.

Means for producing specific hybridization probes for DNAs encoding HSPP include the cloning of polynucleotide sequences encoding HSPP or HSPP derivatives into vectors for the production of mRNA probes. Such vectors are known in the art, are commercially available, and may be used to synthesize RNA probes in vitro by means of 5 the addition of the appropriate RNA polymerases and the appropriate labeled nucleotides. Hybridization probes may be labeled by a variety of reporter groups, for example, by radionuclides such as  $^{32}\text{P}$  or  $^{35}\text{S}$ , or by enzymatic labels, such as alkaline phosphatase coupled to the probe via avidin/biotin coupling systems, and the like.

Polynucleotide sequences encoding HSPP may be used for the diagnosis of 10 disorders associated with expression of HSPP. Examples of such disorders include, but are not limited to, cell proliferative disorders such as actinic keratosis, arteriosclerosis, atherosclerosis, bursitis, cirrhosis, hepatitis, mixed connective tissue disease (MCTD), myelofibrosis, paroxysmal nocturnal hemoglobinuria, polycythemia vera, psoriasis, primary thrombocythemia, and cancers including adenocarcinoma, leukemia, lymphoma, 15 melanoma, myeloma, sarcoma, teratocarcinoma, and, in particular, cancers of the adrenal gland, bladder, bone, bone marrow, brain, breast, cervix, gall bladder, ganglia, gastrointestinal tract, heart, kidney, liver, lung, muscle, ovary, pancreas, parathyroid, penis, prostate, salivary glands, skin, spleen, testis, thymus, thyroid, and uterus; inflammatory disorders, such as acquired immunodeficiency syndrome (AIDS), Addison's 20 disease, adult respiratory distress syndrome, allergies, ankylosing spondylitis, amyloidosis, anemia, asthma, atherosclerosis, autoimmune hemolytic anemia, autoimmune thyroiditis, autoimmune polyenodocrinopathy-candidiasis-ectodermal dystrophy (APECED), bronchitis, cholecystitis, contact dermatitis, Crohn's disease, atopic dermatitis, dermatomyositis, diabetes mellitus, emphysema, episodic lymphopenia with 25 lymphocytotoxins, erythroblastosis fetalis, erythema nodosum, atrophic gastritis, glomerulonephritis, Goodpasture's syndrome, gout, Graves' disease, Hashimoto's thyroiditis, hypereosinophilia, irritable bowel syndrome, multiple sclerosis, myasthenia gravis, myocardial or pericardial inflammation, osteoarthritis, osteoporosis, pancreatitis, polymyositis, psoriasis, Reiter's syndrome, rheumatoid arthritis, scleroderma, Sjögren's 30 syndrome, systemic anaphylaxis, systemic lupus erythematosus, systemic sclerosis, thrombocytopenic purpura, ulcerative colitis, uveitis, Werner syndrome, complications of cancer, hemodialysis, and extracorporeal circulation, viral, bacterial, fungal, parasitic,

protozoal, and helminthic infections, and trauma; cardiovascular disorders including disorders of the blood vessels such as arteriovenous fistula, atherosclerosis, hypertension, vasculitis, Raynaud's disease, aneurysms, arterial dissections, varicose veins, thrombophlebitis and phlebothrombosis, and vascular tumors; disorders of the heart such as congestive heart failure, ischemic heart disease, angina pectoris, myocardial infarction, hypertensive heart disease, degenerative valvular heart disease, calcific aortic valve stenosis, congenitally bicuspid aortic valve, mitral annular calcification, mitral valve prolapse, rheumatic fever and rheumatic heart disease, infective endocarditis, nonbacterial thrombotic endocarditis, endocarditis of systemic lupus erythematosus, carcinoid heart disease, cardiomyopathy, myocarditis, pericarditis, neoplastic heart disease, and congenital heart disease; and disorders of the lungs such as congenital lung anomalies, atelectasis, pulmonary congestion and edema, pulmonary embolism, pulmonary hemorrhage, pulmonary infarction, pulmonary hypertension, vascular sclerosis, obstructive pulmonary disease, restrictive pulmonary disease, chronic obstructive pulmonary disease, emphysema, chronic bronchitis, bronchial asthma, bronchiectasis, bacterial pneumonia, viral and mycoplasmal pneumonia, lung abscess, pulmonary tuberculosis, diffuse interstitial diseases, pneumoconioses, sarcoidosis, idiopathic pulmonary fibrosis, desquamative interstitial pneumonitis, hypersensitivity pneumonitis, pulmonary eosinophilia bronchiolitis obliterans-organizing pneumonia, diffuse pulmonary hemorrhage syndromes, Goodpasture's syndromes, idiopathic pulmonary hemosiderosis, pulmonary involvement in collagen-vascular disorders, pulmonary alveolar proteinosis, lung tumors, inflammatory and noninflammatory pleural effusions, pneumothorax, and pleural tumors; neurological disorders such as epilepsy, ischemic cerebrovascular disease, stroke, cerebral neoplasms, Alzheimer's disease, Pick's disease, Huntington's disease, dementia, Parkinson's disease and other extrapyramidal disorders, amyotrophic lateral sclerosis and other motor neuron disorders, progressive neural muscular atrophy, retinitis pigmentosa, hereditary ataxias, multiple sclerosis and other demyelinating diseases, bacterial and viral meningitis, brain abscess, subdural empyema, epidural abscess, suppurative intracranial thrombophlebitis, myelitis and radiculitis, viral central nervous system disease; prion diseases including kuru, Creutzfeldt-Jakob disease, and Gerstmann-Straussler-Scheinker syndrome; fatal familial insomnia, nutritional and metabolic diseases of the nervous system, neurofibromatosis, tuberous sclerosis, cerebelloretinal

hemangioblastomatosis, encephalotrigeminal syndrome, mental retardation and other developmental disorders of the central nervous system, cerebral palsy, neuroskeletal disorders, autonomic nervous system disorders, cranial nerve disorders, spinal cord diseases, muscular dystrophy and other neuromuscular disorders, peripheral nervous system disorders, dermatomyositis and polymyositis; inherited, metabolic, endocrine, and toxic myopathies; myasthenia gravis, periodic paralysis; mental disorders including mood, anxiety, and schizophrenic disorders; akathesia, amnesia, catatonia, diabetic neuropathy, tardive dyskinesia, dystonias, paranoid psychoses, postherpetic neuralgia, and Tourette's disorder; reproductive disorders such as disorders of prolactin production; infertility, including tubal disease, ovulatory defects, and endometriosis; disruptions of the estrous cycle, disruptions of the menstrual cycle, polycystic ovary syndrome, ovarian hyperstimulation syndrome, endometrial and ovarian tumors, uterine fibroids, autoimmune disorders, ectopic pregnancies, and teratogenesis; cancer of the breast, fibrocystic breast disease, and galactorrhea; disruptions of spermatogenesis, abnormal sperm physiology, cancer of the testis, cancer of the prostate, benign prostatic hyperplasia, prostatitis, Peyronie's disease, carcinoma of the male breast, and gynecomastia; and developmental disorders, such as renal tubular acidosis, anemia, Cushing's syndrome, achondroplastic dwarfism, Duchenne and Becker muscular dystrophy, epilepsy, gonadal dysgenesis, WAGR syndrome (Wilms' tumor, aniridia, genitourinary abnormalities, and mental retardation), Smith-Magenis syndrome, myelodysplastic syndrome, hereditary mucoepithelial dysplasia, hereditary keratodermas, hereditary neuropathies such as Charcot-Marie-Tooth disease and neurofibromatosis, hypothyroidism, hydrocephalus, seizure disorders such as Sydenham's chorea and cerebral palsy, spina bifida, anencephaly, craniorachischisis, congenital glaucoma, cataract, and sensorineural hearing loss. The polynucleotide sequences encoding HSPP may be used in Southern or northern analysis, dot blot, or other membrane-based technologies; in PCR technologies; in dipstick, pin, and multiformat ELISA-like assays; and in microarrays utilizing fluids or tissues from patients to detect altered HSPP expression. Such qualitative or quantitative methods are well known in the art.

In a particular aspect, the nucleotide sequences encoding HSPP may be useful in assays that detect the presence of associated disorders, particularly those mentioned above. The nucleotide sequences encoding HSPP may be labeled by standard methods and added

to a fluid or tissue sample from a patient under conditions suitable for the formation of hybridization complexes. After a suitable incubation period, the sample is washed and the signal is quantitated and compared with a standard value. If the amount of signal in the patient sample is significantly altered in comparison to a control sample then the presence 5 of altered levels of nucleotide sequences encoding HSPP in the sample indicates the presence of the associated disorder. Such assays may also be used to evaluate the efficacy of a particular therapeutic treatment regimen in animal studies, in clinical trials, or to monitor the treatment of an individual patient.

In order to provide a basis for the diagnosis of a disorder associated with 10 expression of HSPP, a normal or standard profile for expression is established. This may be accomplished by combining body fluids or cell extracts taken from normal subjects, either animal or human, with a sequence, or a fragment thereof, encoding HSPP, under conditions suitable for hybridization or amplification. Standard hybridization may be quantified by comparing the values obtained from normal subjects with values from an 15 experiment in which a known amount of a substantially purified polynucleotide is used. Standard values obtained in this manner may be compared with values obtained from samples from patients who are symptomatic for a disorder. Deviation from standard values is used to establish the presence of a disorder.

Once the presence of a disorder is established and a treatment protocol is initiated, 20 hybridization assays may be repeated on a regular basis to determine if the level of expression in the patient begins to approximate that which is observed in the normal subject. The results obtained from successive assays may be used to show the efficacy of treatment over a period ranging from several days to months.

With respect to cancer, the presence of an abnormal amount of transcript (either 25 under- or overexpressed) in biopsied tissue from an individual may indicate a predisposition for the development of the disease, or may provide a means for detecting the disease prior to the appearance of actual clinical symptoms. A more definitive diagnosis of this type may allow health professionals to employ preventative measures or aggressive treatment earlier thereby preventing the development or further progression of 30 the cancer.

Additional diagnostic uses for oligonucleotides designed from the sequences encoding HSPP may involve the use of PCR. These oligomers may be chemically

synthesized, generated enzymatically, or produced *in vitro*. Oligomers will preferably contain a fragment of a polynucleotide encoding HSPP, or a fragment of a polynucleotide complementary to the polynucleotide encoding HSPP, and will be employed under optimized conditions for identification of a specific gene or condition. Oligomers may 5 also be employed under less stringent conditions for detection or quantitation of closely related DNA or RNA sequences.

Methods which may also be used to quantitate the expression of HSPP include radiolabeling or biotinylating nucleotides, coamplification of a control nucleic acid, and interpolating results from standard curves. (See, e.g., Melby, P.C. et al. (1993) *J.*

10 *Immunol. Methods* 159:235-244; Duplaa, C. et al. (1993) *Anal. Biochem.* 212:229-236.) The speed of quantitation of multiple samples may be accelerated by running the assay in an ELISA format where the oligomer of interest is presented in various dilutions and a spectrophotometric or colorimetric response gives rapid quantitation.

In further embodiments, oligonucleotides or longer fragments derived from any of 15 the polynucleotide sequences described herein may be used as targets in a microarray. The microarray can be used to monitor the expression level of large numbers of genes simultaneously and to identify genetic variants, mutations, and polymorphisms. This information may be used to determine gene function, to understand the genetic basis of a disorder, to diagnose a disorder, and to develop and monitor the activities of therapeutic 20 agents.

Microarrays may be prepared, used, and analyzed using methods known in the art. (See, e.g., Brennan, T.M. et al. (1995) U.S. Patent No. 5,474,796; Schena, M. et al. (1996) *Proc. Natl. Acad. Sci.* 93:10614-10619; Baldeschweiler et al. (1995) PCT application WO95/251116; Shalon, D. et al. (1995) PCT application WO95/35505; Heller, R.A. et al. 25 (1997) *Proc. Natl. Acad. Sci.* 94:2150-2155; and Heller, M.J. et al. (1997) U.S. Patent No. 5,605,662.)

In another embodiment of the invention, nucleic acid sequences encoding HSPP may be used to generate hybridization probes useful in mapping the naturally occurring genomic sequence. The sequences may be mapped to a particular chromosome, to a 30 specific region of a chromosome, or to artificial chromosome constructions, e.g., human artificial chromosomes (HACs), yeast artificial chromosomes (YACs), bacterial artificial chromosomes (BACs), bacterial P1 constructions, or single chromosome cDNA libraries.

(See, e.g., Harrington, J.J. et al. (1997) *Nat Genet.* 15:345-355; Price, C.M. (1993) *Blood Rev.* 7:127-134; and Trask, B.J. (1991) *Trends Genet.* 7:149-154.)

Fluorescent in situ hybridization (FISH) may be correlated with other physical chromosome mapping techniques and genetic map data. (See, e.g., Heinz-Ulrich, et al. 5 (1995) in Meyers, *supra*, pp. 965-968.) Examples of genetic map data can be found in various scientific journals or at the Online Mendelian Inheritance in Man (OMIM) site. Correlation between the location of the gene encoding HSPP on a physical chromosomal map and a specific disorder, or a predisposition to a specific disorder, may help define the region of DNA associated with that disorder. The nucleotide sequences of the invention 10 may be used to detect differences in gene sequences among normal, carrier, and affected individuals.

In situ hybridization of chromosomal preparations and physical mapping techniques, such as linkage analysis using established chromosomal markers, may be used for extending genetic maps. Often the placement of a gene on the chromosome of another 15 mammalian species, such as mouse, may reveal associated markers even if the number or arm of a particular human chromosome is not known. New sequences can be assigned to chromosomal arms by physical mapping. This provides valuable information to investigators searching for disease genes using positional cloning or other gene discovery techniques. Once the disease or syndrome has been crudely localized by genetic linkage to 20 a particular genomic region, e.g., ataxia-telangiectasia to 11q22-23, any sequences mapping to that area may represent associated or regulatory genes for further investigation. (See, e.g., Gatti, R.A. et al. (1988) *Nature* 336:577-580.) The nucleotide sequence of the subject invention may also be used to detect differences in the chromosomal location due to translocation, inversion, etc., among normal, carrier, or affected individuals.

25 In another embodiment of the invention, HSPP, its catalytic or immunogenic fragments, or oligopeptides thereof can be used for screening libraries of compounds in any of a variety of drug screening techniques. The fragment employed in such screening may be free in solution, affixed to a solid support, borne on a cell surface, or located intracellularly. The formation of binding complexes between HSPP and the agent being 30 tested may be measured.

Another technique for drug screening provides for high throughput screening of compounds having suitable binding affinity to the protein of interest. (See, e.g., Geysen,

et al. (1984) PCT application WO84/03564.) In this method, large numbers of different small test compounds are synthesized on a solid substrate. The test compounds are reacted with HSPP, or fragments thereof, and washed. Bound HSPP is then detected by methods well known in the art. Purified HSPP can also be coated directly onto plates for use in the 5 aforementioned drug screening techniques. Alternatively, non-neutralizing antibodies can be used to capture the peptide and immobilize it on a solid support.

In another embodiment, one may use competitive drug screening assays in which neutralizing antibodies capable of binding HSPP specifically compete with a test compound for binding HSPP. In this manner, antibodies can be used to detect the 10 presence of any peptide which shares one or more antigenic determinants with HSPP.

In additional embodiments, the nucleotide sequences which encode HSPP may be used in any molecular biology techniques that have yet to be developed, provided the new techniques rely on properties of nucleotide sequences that are currently known, including, but not limited to, such properties as the triplet genetic code and specific base pair 15 interactions.

Without further elaboration, it is believed that one skilled in the art can, using the preceding description, utilize the present invention to its fullest extent. The following preferred specific embodiments are, therefore, to be construed as merely illustrative, and not limitative of the remainder of the disclosure in any way whatsoever.

20 The disclosures of all applications, patents, and publications, mentioned above and below, in particular US Ser. No. 60/090,762, US Ser. No. 60/094,983, US Ser. No. 60/102,686, and US Ser. No. 60/112,129, are hereby expressly incorporated by reference.

## EXAMPLES

25 **I. Construction of cDNA Libraries**

RNA was purchased from Clontech or isolated from tissues described in Table 4. Some tissues were homogenized and lysed in guanidinium isothiocyanate, while others were homogenized and lysed in phenol or in a suitable mixture of denaturants, such as TRIZOL (Life Technologies), a monophasic solution of phenol and guanidine 30 isothiocyanate. The resulting lysates were centrifuged over CsCl cushions or extracted with chloroform. RNA was precipitated from the lysates with either isopropanol or sodium acetate and ethanol, or by other routine methods.

Phenol extraction and precipitation of RNA were repeated as necessary to increase RNA purity. In some cases, RNA was treated with DNase. For most libraries, poly(A+) RNA was isolated using oligo d(T)-coupled paramagnetic particles (Promega), OLIGOTEX latex particles (QIAGEN, Valencia CA), or an OLIGOTEX mRNA 5 purification kit (QIAGEN). Alternatively, RNA was isolated directly from tissue lysates using other RNA isolation kits, e.g., the POLY(A)PURE mRNA purification kit (Ambion, Austin TX).

In some cases, Stratagene was provided with RNA and constructed the corresponding cDNA libraries. Otherwise, cDNA was synthesized and cDNA libraries 10 were constructed with the UNIZAP vector system (Stratagene) or SUPERSCRIPT plasmid system (Life Technologies), using the recommended procedures or similar methods known in the art. (See, e.g., Ausubel, 1997, *supra*, units 5.1-6.6). Reverse transcription was initiated using oligo d(T) or random primers. Synthetic oligonucleotide adapters were ligated to double stranded cDNA, and the cDNA was digested with the appropriate 15 restriction enzyme or enzymes. For most libraries, the cDNA was size-selected (300-1000 bp) using SEPHACRYL S1000, SEPHAROSE CL2B, or SEPHAROSE CL4B column chromatography (Amersham Pharmacia Biotech) or preparative agarose gel electrophoresis. cDNAs were ligated into compatible restriction enzyme sites of the polylinker of a suitable plasmid, e.g., PBLUESCRIPT plasmid (Stratagene), pSPORT1 20 plasmid (Life Technologies), or pINCY (Incyte Pharmaceuticals, Palo Alto CA). Recombinant plasmids were transformed into competent *E. coli* cells including XL1-Blue, XL1-BlueMRF, or SOLR from Stratagene or DH5 $\alpha$ , DH10B, or ElectroMAX DH10B from Life Technologies.

## II. Isolation of cDNA Clones

25 Plasmids were recovered from host cells by *in vivo* excision, using the UNIZAP vector system (Stratagene) or cell lysis. Plasmids were purified using at least one of the following: a MAGIC or WIZARD Minipreps DNA purification system (Promega); an AGTC Miniprep purification kit (Edge Biosystems, Gaithersburg MD); and QIAWELL 8 Plasmid, QIAWELL 8 Plus Plasmid, QIAWELL 8 Ultra Plasmid purification systems or 30 the REAL Prep 96 plasmid kit from QIAGEN. Following precipitation, plasmids were resuspended in 0.1 ml of distilled water and stored, with or without lyophilization, at 4°C.

Alternatively, plasmid DNA was amplified from host cell lysates using direct link PCR in a high-throughput format (Rao, V.B. (1994) *Anal. Biochem.* 216:1-14). Host cell lysis and thermal cycling steps were carried out in a single reaction mixture. Samples were processed and stored in 384-well plates, and the concentration of amplified plasmid 5 DNA was quantified fluorometrically using PICOGREEN dye (Molecular Probes, Eugene OR) and a Fluoroskan II fluorescence scanner (Labsystems Oy, Helsinki, Finland).

### III. Sequencing and Analysis

The cDNAs were prepared for sequencing using the ABI CATALYST 800 (Perkin-Elmer) or the HYDRA microdispenser (Robbins Scientific) or MICROLAB 2200 10 (Hamilton) systems in combination with the PTC-200 thermal cyclers (MJ Research). The cDNAs were sequenced using the ABI PRISM 373 or 377 sequencing systems (Perkin-Elmer) and standard ABI protocols, base calling software, and kits. In one alternative, cDNAs were sequenced using the MEGABACE 1000 DNA sequencing system (Molecular Dynamics). In another alternative, the cDNAs were amplified and sequenced 15 using the ABI PRISM BIGDYE Terminator cycle sequencing ready reaction kit (Perkin-Elmer). In yet another alternative, cDNAs were sequenced using solutions and dyes from Amersham Pharmacia Biotech. Reading frames for the ESTs were determined using standard methods (reviewed in Ausubel, 1997, *supra*, unit 7.7). Some of the cDNA sequences were selected for extension using the techniques disclosed in Example V.

20 The polynucleotide sequences derived from cDNA, extension, and shotgun sequencing were assembled and analyzed using a combination of software programs which utilize algorithms well known to those skilled in the art. Table 5 summarizes the software programs, descriptions, references, and threshold parameters used. The first column of Table 5 shows the tools, programs, and algorithms used, the second column provides a 25 brief description thereof, the third column presents the references which are incorporated by reference herein, and the fourth column presents, where applicable, the scores, probability values, and other parameters used to evaluate the strength of a match between two sequences (the higher the probability the greater the homology). Sequences were analyzed using MACDNASIS PRO software (Hitachi Software Engineering, South San 30 Francisco CA) and LASERGENE software (DNASTAR).

The polynucleotide sequences were validated by removing vector, linker, and polyA sequences and by masking ambiguous bases, using algorithms and programs based

on BLAST, dynamic programing, and dinucleotide nearest neighbor analysis. The sequences were then queried against a selection of public databases such as GenBank primate, rodent, mammalian, vertebrate, and eukaryote databases, and BLOCKS to acquire annotation, using programs based on BLAST, FASTA, and BLIMPS. The sequences were 5 assembled into full length polynucleotide sequences using programs based on Phred, Phrap, and Consed, and were screened for open reading frames using programs based on GeneMark, BLAST, and FASTA. The full length polynucleotide sequences were translated to derive the corresponding full length amino acid sequences, and these full length sequences were subsequently analyzed by querying against databases such as the 10 GenBank databases (described above), SwissProt, BLOCKS, PRINTS, Prosite, and Hidden Markov Model (HMM)-based protein family databases such as PFAM. HMM is a probabilistic approach which analyzes consensus primary structures of gene families. (See, e.g., Eddy, S.R. (1996) Cur. Opin. Str. Biol. 6:361-365.)

15 The programs described above for the assembly and analysis of full length polynucleotide and amino acid sequences were also used to identify polynucleotide sequence fragments from SEQ ID NO:135-268. Fragments from about 20 to about 4000 nucleotides which are useful in hybridization and amplification technologies were described in The Invention section above.

#### IV. Northern Analysis

20 Northern analysis is a laboratory technique used to detect the presence of a transcript of a gene and involves the hybridization of a labeled nucleotide sequence to a membrane on which RNAs from a particular cell type or tissue have been bound. (See, e.g., Sambrook, supra, ch. 7; Ausubel, 1995, supra, ch. 4 and 16.)

25 Analogous computer techniques applying BLAST were used to search for identical or related molecules in nucleotide databases such as GenBank or LIFESEQ database (Incyte Pharmaceuticals). This analysis is much faster than multiple membrane-based hybridizations. In addition, the sensitivity of the computer search can be modified to determine whether any particular match is categorized as exact or similar. The basis of the search is the product score, which is defined as:

30 % sequence identity x % maximum BLAST score

100

The product score takes into account both the degree of similarity between two sequences and the length of the sequence match. For example, with a product score of 40, the match will be exact within a 1% to 2% error, and, with a product score of 70, the match will be exact. Similar molecules are usually identified by selecting those which show product 5 scores between 15 and 40, although lower scores may identify related molecules.

The results of northern analyses are reported as a percentage distribution of libraries in which the transcript encoding HSPP occurred. Analysis involved the categorization of cDNA libraries by organ/tissue and disease. The organ/tissue categories included cardiovascular, dermatologic, developmental, endocrine, gastrointestinal, 10 hematopoietic/immune, musculoskeletal, nervous, reproductive, and urologic. The disease/condition categories included cancer, inflammation/trauma, cell proliferation, neurological, and pooled. For each category, the number of libraries expressing the sequence of interest was counted and divided by the total number of libraries across all categories. Percentage values of tissue-specific and disease- or condition-specific 15 expression are reported in Table 3.

#### V. Extension of HSPP Encoding Polynucleotides

Full length nucleic acid sequences of SEQ ID NOs:135-229 were produced by extension of the component fragments described in Table 1, column 5, using oligonucleotide primers based on these fragments. For each nucleic acid sequence, one 20 primer was synthesized to initiate extension of an antisense polynucleotide, and the other was synthesized to initiate extension of a sense polynucleotide. Primers were used to facilitate the extension of the known sequence "outward" generating amplicons containing new unknown nucleotide sequence for the region of interest. The initial primers were designed from the cDNA using OLIGO™ 4.06 (National Biosciences, Plymouth, MN), or 25 another appropriate program, to be about 22 to 30 nucleotides in length, to have a GC content of about 50% or more, and to anneal to the target sequence at temperatures of about 68°C to about 72°C. Any stretch of nucleotides which would result in hairpin structures and primer-primer dimerizations was avoided.

Selected human cDNA libraries (GIBCO BRL) were used to extend the sequence.

30 If more than one extension is necessary or desired, additional sets of primers are designed to further extend the known region.

High fidelity amplification was obtained by following the instructions for the XL-PCR™ kit (The Perkin-Elmer Corp., Norwalk, CT) and thoroughly mixing the enzyme and reaction mix. PCR was performed using the PTC-200 thermal cycler (MJ Research, Inc., Watertown, MA), beginning with 40 pmol of each primer and the recommended

5 concentrations of all other components of the kit, with the following parameters:

Step 1	94° C for 1 min (initial denaturation)
Step 2	65° C for 1 min
Step 3	68° C for 6 min
Step 4	94° C for 15 sec
10 Step 5	65° C for 1 min
Step 6	68° C for 7 min
Step 7	Repeat steps 4 through 6 for an additional 15 cycles
Step 8	94° C for 15 sec
Step 9	65° C for 1 min
15 Step 10	68° C for 7:15 min
Step 11	Repeat steps 8 through 10 for an additional 12 cycles
Step 12	72° C for 8 min
Step 13	4° C (and holding)

20 A 5  $\mu$ l to 10  $\mu$ l aliquot of the reaction mixture was analyzed by electrophoresis on a low concentration (about 0.6% to 0.8%) agarose mini-gel to determine which reactions were successful in extending the sequence. Bands thought to contain the largest products were excised from the gel, purified using QIAQUICK™ (QIAGEN Inc.), and trimmed of overhangs using Klenow enzyme to facilitate religation and cloning.

25 After ethanol precipitation, the products were redissolved in 13  $\mu$ l of ligation buffer, 1  $\mu$ l T4-DNA ligase (15 units) and 1  $\mu$ l T4 polynucleotide kinase were added, and the mixture was incubated at room temperature for 2 to 3 hours, or overnight at 16° C. Competent E. coli cells (in 40  $\mu$ l of appropriate media) were transformed with 3  $\mu$ l of ligation mixture and cultured in 80  $\mu$ l of SOC medium. (See, e.g., Sambrook, supra,

30 Appendix A, p. 2.) After incubation for one hour at 37° C, the E. coli mixture was plated on Luria Bertani (LB) agar (See, e.g., Sambrook, supra, Appendix A, p. 1) containing carbenicillin (2x carb). The following day, several colonies were randomly picked from each plate and cultured in 150  $\mu$ l of liquid LB/2x carb medium placed in an individual well of an appropriate commercially-available sterile 96-well microtiter plate. The 35 following day, 5  $\mu$ l of each overnight culture was transferred into a non-sterile 96-well plate and, after dilution 1:10 with water, 5  $\mu$ l from each sample was transferred into a PCR array.

For PCR amplification, 18  $\mu$ l of concentrated PCR reaction mix (3.3x) containing 4 units of rTth DNA polymerase, a vector primer, and one or both of the gene specific primers used for the extension reaction were added to each well. Amplification was performed using the following conditions:

5	Step 1	94° C for 60 sec
	Step 2	94° C for 20 sec
	Step 3	55° C for 30 sec
	Step 4	72° C for 90 sec
	Step 5	Repeat steps 2 through 4 for an additional 29 cycles
10	Step 6	72° C for 180 sec
	Step 7	4° C (and holding)

Aliquots of the PCR reactions were run on agarose gels together with molecular weight markers. The sizes of the PCR products were compared to the original partial 15 cDNAs, and appropriate clones were selected, ligated into plasmid, and sequenced.

The full length nucleic acid sequences of SEQ ID NO:230-268 were produced by extension of an appropriate fragment of the full length molecule using oligonucleotide primers designed from this fragment. One primer was synthesized to initiate 5' extension of the known fragment, and the other primer, to initiate 3' extension of the known 20 fragment. The initial primers were designed using OLIGO 4.06 software (National Biosciences), or another appropriate program, to be about 22 to 30 nucleotides in length, to have a GC content of about 50% or more, and to anneal to the target sequence at temperatures of about 68° C to about 72° C. Any stretch of nucleotides which would result in hairpin structures and primer-primer dimerizations was avoided.

25 Selected human cDNA libraries were used to extend the sequence. If more than one extension was necessary or desired, additional or nested sets of primers were designed.

High fidelity amplification was obtained by PCR using methods well known in the art. PCR was performed in 96-well plates using the PTC-200 thermal cycler (MJ Research, Inc.). The reaction mix contained DNA template, 200 nmol of each primer, 30 reaction buffer containing Mg<sup>2+</sup>, (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, and  $\beta$ -mercaptoethanol, Taq DNA polymerase (Amersham Pharmacia Biotech), ELONGASE enzyme (Life Technologies), and Pfu DNA polymerase (Stratagene), with the following parameters for primer pair PCI A and PCI B: Step 1: 94° C, 3 min; Step 2: 94° C, 15 sec; Step 3: 60° C, 1 min; Step 4: 68° C, 2 min; Step 5: Steps 2, 3, and 4 repeated 20 times; Step 6: 68° C, 5 min; Step 7: 35 storage at 4° C. In the alternative, the parameters for primer pair T7 and SK+ were as

follows: Step 1: 94°C, 3 min; Step 2: 94°C, 15 sec; Step 3: 57°C, 1 min; Step 4: 68°C, 2 min; Step 5: Steps 2, 3, and 4 repeated 20 times; Step 6: 68°C, 5 min; Step 7: storage at 4°C.

The concentration of DNA in each well was determined by dispensing 100 µl 5 PICOGREEN quantitation reagent (0.25% (v/v) PICOGREEN; Molecular Probes, Eugene OR) dissolved in 1X TE and 0.5 µl of undiluted PCR product into each well of an opaque fluorimeter plate (Corning Costar, Acton MA), allowing the DNA to bind to the reagent. The plate was scanned in a Fluoroskan II (Labsystems Oy, Helsinki, Finland) to measure 10 the fluorescence of the sample and to quantify the concentration of DNA. A 5 µl to 10 µl aliquot of the reaction mixture was analyzed by electrophoresis on a 1 % agarose mini-gel to determine which reactions were successful in extending the sequence.

The extended nucleotides were desalted and concentrated, transferred to 384-well plates, digested with CviJI cholera virus endonuclease (Molecular Biology Research, Madison WI), and sonicated or sheared prior to religation into pUC 18 vector (Amersham 15 Pharmacia Biotech). For shotgun sequencing, the digested nucleotides were separated on low concentration (0.6 to 0.8%) agarose gels, fragments were excised, and agar digested with Agar ACE (Promega). Extended clones were religated using T4 ligase (New England Biolabs, Beverly MA) into pUC 18 vector (Amersham Pharmacia Biotech), treated with Pfu DNA polymerase (Stratagene) to fill-in restriction site overhangs, and transfected into 20 competent E. coli cells. Transformed cells were selected on antibiotic-containing media, individual colonies were picked and cultured overnight at 37°C in 384-well plates in LB/2x carb liquid media.

The cells were lysed, and DNA was amplified by PCR using Taq DNA 25 polymerase (Amersham Pharmacia Biotech) and Pfu DNA polymerase (Stratagene) with the following parameters: Step 1: 94°C, 3 min; Step 2: 94°C, 15 sec; Step 3: 60°C, 1 min; Step 4: 72°C, 2 min; Step 5: steps 2, 3, and 4 repeated 29 times; Step 6: 72°C, 5 min; Step 7: storage at 4°C. DNA was quantified by PICOGREEN reagent (Molecular Probes) as described above. Samples with low DNA recoveries were reamplified using the same conditions as described above. Samples were diluted with 20% dimethylsulphoxide (1:2, 30 v/v), and sequenced using DYENAMIC energy transfer sequencing primers and the DYENAMIC DIRECT kit (Amersham Pharmacia Biotech) or the ABI PRISM BIGDYE Terminator cycle sequencing ready reaction kit (Perkin-Elmer).

In like manner, the nucleotide sequences of SEQ ID NO:135-268 are used to obtain 5' regulatory sequences using the procedure above, oligonucleotides designed for such extension, and an appropriate genomic library.

5 **VI. Labeling and Use of Individual Hybridization Probes**

Hybridization probes derived from SEQ ID NO:135-268 are employed to screen cDNAs, genomic DNAs, or mRNAs. Although the labeling of oligonucleotides, consisting of about 20 base pairs, is specifically described, essentially the same procedure is used with larger nucleotide fragments. Oligonucleotides are designed using state-of-the-  
10 art software such as OLIGO 4.06 software (National Biosciences) and labeled by combining 50 pmol of each oligomer, 250  $\mu$ Ci of [ $\gamma$ -<sup>32</sup>P] adenosine triphosphate (Amersham Pharmacia Biotech), and T4 polynucleotide kinase (DuPont NEN, Boston MA). The labeled oligonucleotides are substantially purified using a SEPHADEX G-25 superfine size exclusion dextran bead column (Amersham Pharmacia Biotech). An aliquot  
15 containing  $10^7$  counts per minute of the labeled probe is used in a typical membrane-based hybridization analysis of human genomic DNA digested with one of the following endonucleases: Ase I, Bgl II, Eco RI, Pst I, Xba1, or Pvu II (DuPont NEN).

The DNA from each digest is fractionated on a 0.7% agarose gel and transferred to nylon membranes (Nytran Plus, Schleicher & Schuell, Durham NH). Hybridization is  
20 carried out for 16 hours at 40°C. To remove nonspecific signals, blots are sequentially washed at room temperature under increasingly stringent conditions up to 0.1 x saline sodium citrate and 0.5% sodium dodecyl sulfate. After XOMAT-AR film (Eastman Kodak, Rochester NY) is exposed to the blots to film for several hours, hybridization patterns are compared visually.

25 **VII. Microarrays**

A chemical coupling procedure and an ink jet device can be used to synthesize array elements on the surface of a substrate. (See, e.g., Baldeschweiler, supra.) An array analogous to a dot or slot blot may also be used to arrange and link elements to the surface of a substrate using thermal, UV, chemical, or mechanical bonding procedures. A typical  
30 array may be produced by hand or using available methods and machines and contain any appropriate number of elements. After hybridization, nonhybridized probes are removed and a scanner used to determine the levels and patterns of fluorescence. The degree of

complementarity and the relative abundance of each probe which hybridizes to an element on the microarray may be assessed through analysis of the scanned images.

Full-length cDNAs, Expressed Sequence Tags (ESTs), or fragments thereof may comprise the elements of the microarray. Fragments suitable for hybridization can be 5 selected using software well known in the art such as LASERGENE software (DNASTAR). Full-length cDNAs, ESTs, or fragments thereof corresponding to one of the nucleotide sequences of the present invention, or selected at random from a cDNA library relevant to the present invention, are arranged on an appropriate substrate, e.g., a glass slide. The cDNA is fixed to the slide using, e.g., UV cross-linking followed by thermal 10 and chemical treatments and subsequent drying. (See, e.g., Schena, M. et al. (1995) *Science* 270:467-470; Shalon, D. et al. (1996) *Genome Res.* 6:639-645.) Fluorescent probes are prepared and used for hybridization to the elements on the substrate. The substrate is analyzed by procedures described above.

### **VIII. Complementary Polynucleotides**

15 Sequences complementary to the HSPP-encoding sequences, or any parts thereof, are used to detect, decrease, or inhibit expression of naturally occurring HSPP. Although use of oligonucleotides comprising from about 15 to 30 base pairs is described, essentially the same procedure is used with smaller or with larger sequence fragments. Appropriate oligonucleotides are designed using OLIGO 4.06 software (National Biosciences) and the 20 coding sequence of HSPP. To inhibit transcription, a complementary oligonucleotide is designed from the most unique 5' sequence and used to prevent promoter binding to the coding sequence. To inhibit translation, a complementary oligonucleotide is designed to prevent ribosomal binding to the HSPP-encoding transcript.

### **25 IX. Expression of HSPP**

Expression and purification of HSPP is achieved using bacterial or virus-based expression systems. For expression of HSPP in bacteria, cDNA is subcloned into an appropriate vector containing an antibiotic resistance gene and an inducible promoter that directs high levels of cDNA transcription. Examples of such promoters include, but are 30 not limited to, the *trp-lac* (*tac*) hybrid promoter and the T5 or T7 bacteriophage promoter in conjunction with the *lac* operator regulatory element. Recombinant vectors are transformed into suitable bacterial hosts, e.g., BL21(DE3). Antibiotic resistant bacteria

express HSPP upon induction with isopropyl beta-D-thiogalactopyranoside (IPTG). Expression of HSPP in eukaryotic cells is achieved by infecting insect or mammalian cell lines with recombinant Autographica californica nuclear polyhedrosis virus (AcMNPV), commonly known as baculovirus. The nonessential polyhedrin gene of baculovirus is 5 replaced with cDNA encoding HSPP by either homologous recombination or bacterial-mediated transposition involving transfer plasmid intermediates. Viral infectivity is maintained and the strong polyhedrin promoter drives high levels of cDNA transcription. Recombinant baculovirus is used to infect Spodoptera frugiperda (Sf9) insect cells in most cases, or human hepatocytes, in some cases. Infection of the latter requires additional 10 genetic modifications to baculovirus. (See Engelhard, E. K. et al. (1994) Proc. Natl. Acad. Sci. USA 91:3224-3227; Sandig, V. et al. (1996) Hum. Gene Ther. 7:1937-1945.)

In most expression systems, HSPP is synthesized as a fusion protein with, e.g., glutathione S-transferase (GST) or a peptide epitope tag, such as FLAG or 6-His, permitting rapid, single-step, affinity-based purification of recombinant fusion protein 15 from crude cell lysates. GST, a 26-kilodalton enzyme from Schistosoma japonicum, enables the purification of fusion proteins on immobilized glutathione under conditions that maintain protein activity and antigenicity (Amersham Pharmacia Biotech). Following purification, the GST moiety can be proteolytically cleaved from HSPP at specifically engineered sites. FLAG, an 8-amino acid peptide, enables immunoaffinity purification 20 using commercially available monoclonal and polyclonal anti-FLAG antibodies (Eastman Kodak). 6-His, a stretch of six consecutive histidine residues, enables purification on metal-chelate resins (QIAGEN). Methods for protein expression and purification are discussed in Ausubel (1995, supra, ch 10 and 16). Purified HSPP obtained by these methods can be used directly in the following activity assay.

25

## **X. Demonstration of HSPP Activity**

### **HSPP-68**

30 HSPP-68 activity is measured by determining the potassium current using voltage clamp analysis on single Xenopus laevis oocytes injected with HSPP-68 cRNA. HSPP-68 cRNA is synthesized in vitro from linearized HSPP-68 encoding plasmids using the T7

RNA polymerase and injected into oocytes.. Injected oocytes are used two to four days after injection. In a 0.3 ml perfusion chamber, a single oocyte is impaled with two standard microelectrodes (1-2.5 M $\Omega$ ) filled with 3 M KCl. The oocyte is maintained under voltage clamp by using a Dagan TEV 200 amplifier, in buffer containing 96 mM 5 NaCl, 2 mM KCl, 1.8 mM CaCl<sub>2</sub>, 2 mM MgCl<sub>2</sub>, 5 mM HEPES, pH 7.4 with NaOH. Stimulation of the preparation, data acquisition, and analysis is performed using a computer. All experiments are performed at room temperature (21-22 °C). Following a depolarizing pulse, the characteristics of the resulting potassium current are measured via the recording electrode. The amount of potassium current that flows in response to a unit 10 depolarization is proportional to the activity of HSPP-68 in the cell. (Duprat, F. et al. (1997) EMBO J. 16:5464-5471.)

#### HSPP-92

HSPP-92 protein phosphatase activity is measured by the hydrolysis of P-nitrophenyl phosphate (PNPP). HSPP-92 is incubated together with PNPP in HEPES 15 buffer pH 7.5, in the presence of 0.1% *b*-mercaptoethanol at 37°C for 60 min. The reaction is stopped by the addition of 6 ml of 10 N NaOH and the increase in light absorbance at 410 nm resulting from the hydrolysis of PNPP is measured using a spectrophotometer. The increase in light absorbance is proportional to the activity of PP in the assay. (Diamond R.H. et al (1994) Mol Cell Biol 14:3752-62.)

20 Alternatively, HSPP, or biologically active fragments thereof, are labeled with <sup>125</sup>I Bolton-Hunter reagent. (See, e.g., Bolton et al. (1973) Biochem. J. 133:529.) Candidate molecules previously arrayed in the wells of a multi-well plate are incubated with the labeled HSPP, washed, and any wells with labeled HSPP complex are assayed. Data obtained using different concentrations of HSPP are used to calculate values for the 25 number, affinity, and association of HSPP with the candidate molecules.

Alternatively, an assay for HSPP activity measures the expression of HSPP on the cell surface. cDNA encoding HSPP is subcloned into an appropriate mammalian expression vector suitable for high levels of cDNA expression. The resulting construct is transfected into a nonhuman cell line such as NIH3T3. Cell surface proteins are labeled 30 with biotin using methods known in the art. Immunoprecipitations are performed using HSPP-specific antibodies, and immunoprecipitated samples are analyzed using SDS-PAGE and immunoblotting techniques. The ratio of labeled immunoprecipitant to

unlabeled immunoprecipitant is proportional to the amount of HSPP expressed on the cell surface.

Alternatively, an assay for HSPP activity measures the amount of HSPP in secretory, membrane-bound organelles. Transfected cells as described above are harvested 5 and lysed. The lysate is fractionated using methods known to those of skill in the art, for example, sucrose gradient ultracentrifugation. Such methods allow the isolation of subcellular components such as the Golgi apparatus, ER, small membrane-bound vesicles, and other secretory organelles. Immunoprecipitations from fractionated and total cell lysates are performed using HSPP-specific antibodies, and immunoprecipitated samples 10 are analyzed using SDS-PAGE and immunoblotting techniques. The concentration of HSPP in secretory organelles relative to HSPP in total cell lysate is proportional to the amount of HSPP in transit through the secretory pathway.

## XI. Functional Assays

HSPP function is assessed by expressing the sequences encoding HSPP at 15 physiologically elevated levels in mammalian cell culture systems. cDNA is subcloned into a mammalian expression vector containing a strong promoter that drives high levels of cDNA expression. Vectors of choice include pCMV SPORT (Life Technologies) and pCR3.1 (Invitrogen, Carlsbad CA), both of which contain the cytomegalovirus promoter. 5-10  $\mu$ g of recombinant vector are transiently transfected into a human cell line, preferably 20 of endothelial or hematopoietic origin, using either liposome formulations or electroporation. 1-2  $\mu$ g of an additional plasmid containing sequences encoding a marker protein are co-transfected. Expression of a marker protein provides a means to distinguish transfected cells from nontransfected cells and is a reliable predictor of cDNA expression from the recombinant vector. Marker proteins of choice include, e.g., Green Fluorescent 25 Protein (GFP; Clontech), CD64, or a CD64-GFP fusion protein. Flow cytometry (FCM), an automated, laser optics-based technique, is used to identify transfected cells expressing GFP or CD64-GFP, and to evaluate properties, for example, their apoptotic state. FCM detects and quantifies the uptake of fluorescent molecules that diagnose events preceding or coincident with cell death. These events include changes in nuclear DNA content as 30 measured by staining of DNA with propidium iodide; changes in cell size and granularity as measured by forward light scatter and 90 degree side light scatter; down-regulation of DNA synthesis as measured by decrease in bromodeoxyuridine uptake; alterations in

expression of cell surface and intracellular proteins as measured by reactivity with specific antibodies; and alterations in plasma membrane composition as measured by the binding of fluorescein-conjugated Annexin V protein to the cell surface. Methods in flow cytometry are discussed in Ormerod, M. G. (1994) Flow Cytometry, Oxford, New York

5 NY.

The influence of HSPP on gene expression can be assessed using highly purified populations of cells transfected with sequences encoding HSPP and either CD64 or CD64-GFP. CD64 and CD64-GFP are expressed on the surface of transfected cells and bind to conserved regions of human immunoglobulin G (IgG). Transfected cells are efficiently 10 separated from nontransfected cells using magnetic beads coated with either human IgG or antibody against CD64 (DYNAL, Lake Success NY). mRNA can be purified from the cells using methods well known by those of skill in the art. Expression of mRNA encoding HSPP and other genes of interest can be analyzed by northern analysis or microarray techniques.

15 **XII. Production of HSPP Specific Antibodies**

HSPP substantially purified using polyacrylamide gel electrophoresis (PAGE; see, e.g., Harrington, M.G. (1990) *Methods Enzymol.* 182:488-495), or other purification techniques, is used to immunize rabbits and to produce antibodies using standard protocols.

20 Alternatively, the HSPP amino acid sequence is analyzed using LASERGENE software (DNASTAR) to determine regions of high immunogenicity, and a corresponding oligopeptide is synthesized and used to raise antibodies by means known to those of skill in the art. Methods for selection of appropriate epitopes, such as those near the C-terminus or in hydrophilic regions are well described in the art. (See, e.g., Ausubel, 1995, supra, ch. 11.)

25 Typically, oligopeptides 15 residues in length are synthesized using an ABI 431A Peptide Synthesizer (Perkin-Elmer) using fmoc-chemistry and coupled to KLH (Sigma-Aldrich, St. Louis MO) by reaction with N-maleimidobenzoyl-N-hydroxysuccinimide ester (MBS) to increase immunogenicity. (See, e.g., Ausubel, 1995, supra.) Rabbits are 30 immunized with the oligopeptide-KLH complex in complete Freund's adjuvant. Resulting antisera are tested for antipeptide activity by, for example, binding the peptide to plastic,

blocking with 1% BSA, reacting with rabbit antisera, washing, and reacting with radio-iodinated goat anti-rabbit IgG.

### XIII. Purification of Naturally Occurring HSPP Using Specific Antibodies

Naturally occurring or recombinant HSPP is substantially purified by

- 5 immunoaffinity chromatography using antibodies specific for HSPP. An immunoaffinity column is constructed by covalently coupling anti-HSPP antibody to an activated chromatographic resin, such as CNBr-activated SEPHAROSE (Amersham Pharmacia Biotech). After the coupling, the resin is blocked and washed according to the manufacturer's instructions.
- 10 Media containing HSPP are passed over the immunoaffinity column, and the column is washed under conditions that allow the preferential absorbance of HSPP (e.g., high ionic strength buffers in the presence of detergent). The column is eluted under conditions that disrupt antibody/HSPP binding (e.g., a buffer of pH 2 to pH 3, or a high concentration of a chaotrope, such as urea or thiocyanate ion), and HSPP is collected.

### XIV. Identification of Molecules Which Interact with HSPP

HSPP, or biologically active fragments thereof, are labeled with  $^{125}\text{I}$  Bolton-Hunter reagent. (See, e.g., Bolton et al. (1973) Biochem. J. 133:529.) Candidate molecules previously arrayed in the wells of a multi-well plate are incubated with the labeled HSPP, washed, and any wells with labeled HSPP complex are assayed. Data obtained using different concentrations of HSPP are used to calculate values for the number, affinity, and association of HSPP with the candidate molecules.

Various modifications and variations of the described methods and systems of the invention will be apparent to those skilled in the art without departing from the scope and spirit of the invention. Although the invention has been described in connection with specific preferred embodiments, it should be understood that the invention as claimed should not be unduly limited to such specific embodiments. Indeed, various modifications of the described modes for carrying out the invention which are obvious to those skilled in molecular biology or related fields are intended to be within the scope of the following claims.

TABLE 1

Protein SEQ ID NO:	Nucleotide SEQ ID NO:	Clone ID	Library	Fragments
1	135	443531	MPHGNOT03	443531H1 (MPHGNOT03), 1406807F6 (LATRTUT02), 443531T6 (MPHGNOT03), SBBA00451F1, SBBA00676F1
2	136	632860	NEUTGMT01	632860H1 (NEUTGMT01), 784715R3 (PROSNOT05), 509590H1 (MPHGNOT03)
3	137	670010	CRBLNOT01	670010H1 (CRBLNOT01), 669971R1 (CRBLNOT01), 1553045F1 (BLADTUT04)
4	138	726498	SYNOOAT01	726498H1 (SYNOOAT01), 726498R6 (SYNOOAT01), 866599R3 (BRAITUT03)
5	139	795064	OVARNOT03	795064H1 (OVARNOT03), 4339458H1 (BRAUNOT02), 937605R3 (CERVNNOT01), 2381151F6 (ISLTNOT01), 1466346F6 (PANCTUT02)
6	140	924925	BRAINOT04	924925H1 (BRAINOT04), 3268330H1 (BRAINOT20), 759120R3 (BRAITUT02)
7	141	962390	BRSTTUT03	962390H1 (BRSTTUT03), 1907958F6 (CONNNTUT01), 0235569F1 (ADENINB01), 167282F1 (LIVRNOT01), 1309211F1 (COLNFET02), SAUA00696F1, SAUA02860F1
8	142	1259405	MENITUT03	1259405H1 (MENITUT03), 2472425H1 (THPINOT03), 774303R1 (COLNNNOT05), 1520779F1 (BLADTUT04), 1693833F6 (COLNNNOT23), 1831858T6.comp (THPIAZT01), 1527737T6.comp (UCMCL5T01)
9	143	1297384	BRSTNOT07	1297384H1 (BRSTNOT07), 1269310F6 (BRAINOT09), 1457367F1 (COLNFET02), 415587R1 (BRSTNOT01), SAN02967F1
10	144	1299627	BRSTNOT07	1299627H1 (BRSTNOT07), 1359140F6 (LUNGNOT09), 1349224F1 (LATRTUT02), SBAA01431F1, SBAA02909F1, SBAA01156F1
11	145	1306026	PLACNOT02	1306026H1 (PLACNOT02), 1464088R6 (PANCNOT04), SBAA02496F1, SBAA04305F1
12	146	1316219	BLADTUT02	1316219H1 (BLADTUT02), 2458603F6 (ENDANOT01), 2504756T6 (CONUTUT01)
13	147	1329031	PANCNOT07	1329031H1 (PANCNOT07), 1329031T6 (PANCNOT07), 1329031F6 (PANCNOT07)

TABLE 1 (cont.)

Protein SEQ ID NO:	Nucleotide SEQ ID NO:	Clone ID	Library	Fragments	
				PROSNON01	(THPIAZS08)
85	219	2241736	PANCTUT02	833263H1 (PROSTUT04), 2241736H1 (PANCTUT02), SAZA01148F1, SASA03299F1, SASA01349F1	
86	220	2271935		2271935H1 (PROSNON01), 2276774H1 (PROSNON01), 2760171T6	
87	221	2295344	BRSTNOT05	2295344H1 (BRSTNOT05), 3288561F6 (BONRFET01), SBCGA01801F1	
88	222	2303994	BRSTNOT05	905482T1 (COLNNOT08), 1858636F6 (PROSNOT18), 2303994H1 (BRSTNOT05)	
89	223	2497805	ADRETUT05	2497805F6 (ADRETUT05), 2497805H1 (ADRETUT05)	
90	224	2646362	LUNGUT11	1754702H1 (LIVRTUT01), 2640776T6 (LUNGUT08), 2646362H1 (LUNGUT11), 3356773H1 (PROSTUT16)	
91	225	2657146	LUNGUT09	2657146F6 (LUNGUT09), 2657146H1 (LUNGUT09)	
92	226	2755786	THPIAZS08	288436R1 (EOSIHE02), 1252824F6 (LUNGFE03), 1305549H1 (PLACNOT02), 1364975R1 (SCORN02), 2018293H1 (THPINOT01), 2047320H1 (THPIT7T01), 2184537F6 (SININOT01), 2755786H1 (THPIAZS08), 4111022H1 (PROSBPT07)	
93	227	2831245	TLYMN0T03	2831245H1 (TLYMN0T03), SBMA01396F1	
94	228	3116250	LUNGUT13	126263F1 (LUNGNOT01), 2729942H1 (OVARTUT04), 3116250H1 (LUNGUT13)	
95	229	3129630	LUNGUT12	3129630F6 (LUNGUT12), 3129630H1 (LUNGUT12), SBDA06436F1	
96	230	0076332	HMCINOT01	0076332H1 (HMCINOT01), 0076332R6 (HMCINOT01), 007632T6 (HMCINOT01)	
97	231	1236368	LUNGFE03	123668H1 (LUNGFE03), SBAA02713F1, SBAA03203F1, SBAA04196F1	
98	232	1334153	COLNNOT13	776410R1 (COLNNOT05), 1334153H1 (COLNNOT13), 1334153T1 (COLNNOT13), 1800085F6 (COLNNOT27), 2701948H1 (OVARTUT0)	

TABLE 2 (cont.)

Protein SEQ ID NO:	Amino Acid Residues	Potential Phosphorylation Sites	Potential Glycosylation Sites	Signature Sequences	Identification	Analytical Methods
95	128	S91		M1 through about G31 Transmembrane: about F108 through about L126		SPScan HMM MOTIFS
96	124	T115 T43 S91		M1-S20  P116-V124 (urotensin II signature)		SPScan HMM Motifs BLOCKS BLAST
97	182	S28 T70 S172 S25 S32 S48 S108 S131		M1-S23, M1-S25		SPScan HMM Motifs
98	237	S55 S88 S121 S135	N45 N73 N107 N118 N132 N172 N175 N185	M1-A16, M1-S21  C40-C198 (cysteine spacing pattern similar to that of RoBo-1)		SPScan HMM Motifs BLOCKS BLAST
99	160	S36 S59 T143		M1-A27		
100	148	T76 S64 Y103		M1-S30, M1-G31		SPScan HMM Motifs
101	170	S78 T4 T30 S130 S25 S29 T122		M1-A23, M1-L28		SPScan HMM Motifs

TABLE 3 (cont.)

Nucleotide SEQ ID NO:	Tissue Expression (Fraction of Total)	Disease/Condition-Specific Expression (Total of Fraction)	Vector
227	Lymphocytes (1.000)	Inflammation (1.000)	pINCY
228	Cardiovascular (0.531) Reproductive (0.250) Urologic (0.094)	Cancer (0.656) Inflammation (0.250) Fetal or Proliferating (0.094)	pINCY
229	Reproductive (0.333) Cardiovascular (0.167) Gastrointestinal (0.167) Endocrine (0.167) Hematopoietic/Immune (0.167)	Cancer (0.500) Fetal or Proliferating (0.167) Inflammation (0.333)	pINCY
230	Hematopoietic/Immune (0.500) Reproductive (0.500)	Cell Proliferation (0.500) Inflammation (0.500)	pBLUESCRIPT
231	Cardiovascular (0.333) Nervous (0.333) Developmental (0.167)	Cancer (0.500) Cell Proliferation (0.333) Inflammation (0.167)	pINCY
232	Gastrointestinal (0.938) Reproductive (0.062)	Cancer (0.500) Inflammation (0.500)	pINCY
233	Nervous (0.324) Reproductive (0.235) Hematopoietic/Immune (0.118)	Cancer (0.456) Inflammation (0.235) Trauma (0.147)	pINCY
234	Nervous (0.255) Reproductive (0.255) Musculoskeletal (0.182)	Cancer (0.545) Inflammation (0.255) Trauma (0.109)	pINCY
235	Musculoskeletal (0.308) Reproductive (0.231) Gastrointestinal (0.154)	Cancer (0.538) Inflammation (0.231) Trauma (0.154)	pINCY
236	Nervous (1.000)	Cancer (1.000)	pINCY
237	Gastrointestinal (0.429) Hematopoietic/Immune (0.143) Nervous (0.143)	Cancer (0.571) Cell Proliferation (0.143) Trauma (0.143)	pINCY
238	Reproductive (0.254) Gastrointestinal (0.160) Nervous (0.128)	Cancer (0.453) Inflammation (0.241) Cell Proliferation (0.175)	pINCY
239	Nervous (0.333) Dermatologic (0.167) Endocrine (0.167)	Trauma (0.333) Cancer (0.167) Cell Proliferation (0.167)	pINCY

TABLE 4 (cont.)

Polynucleotide SEQ ID NO:	Clone ID	Library	Library Description
226	2755786	THP1AZS08	This subtracted THP-1 promonocyte cell line library was constructed using 5.76 million clones from a 5-aza-2'-deoxycytidine (AZ) treated THP-1 cell library. Starting RNA was made from THP-1 promonocyte cells treated for three days with 0.8 micromolar AZ. The hybridization probe for subtraction was derived from a similarly constructed library, made from RNA isolated from untreated THP-1 cells. 5.76 million clones from the AZ-treated THP-1 cell library were then subjected to two rounds of subtractive hybridization with 5 million clones from the untreated THP-1 cell library. Subtractive hybridization conditions were based on the methodologies of Swaroop et al., NAR (1991) 19:1954, and Bonaldo et al., Genome Research (1996) 6:791. THP-1 (ATCC TIB-202) is a human promonocyte line derived from peripheral blood of a 1-year-old Caucasian male with acute monocytic leukemia.
227	2831245	TLYMNOT03	The library was constructed using RNA isolated from nonactivated Th1 cells. These cells were differentiated from umbilical cord CD4 T cells with IL-12 and B7-transfected COS cells.
228	3116250	LUNGUTUT13	The library was constructed using RNA isolated from tumorous lung tissue removed from the right upper lobe of a 47-year-old Caucasian male during a segmental lung resection. Pathology indicated invasive grade 3 (of 4) adenocarcinoma. Family history included atherosclerotic coronary artery disease, and type II diabetes.
229	3129630	LUNGUTUT12	The library was constructed using RNA isolated from tumorous lung tissue removed from a 70-year-old Caucasian female during a lung lobectomy of the left upper lobe. Pathology indicated grade 3 (of 4) adenocarcinoma and vascular invasion. Patient history included tobacco abuse, depressive disorder, anxiety state, and skin cancer. Family history included cerebrovascular disease, congestive heart failure, colon cancer, depressive disorder, and primary liver.
230	007632	HMC1NOT01	The library was constructed using RNA isolated from the HMC-1 human mast cell line derived from a 52-year-old female. Patient history included mast cell leukemia.
231	1236968	LUNGFFET03	The library was constructed using RNA isolated from lung tissue removed from a Caucasian female fetus who died at 20 weeks' gestation.
232	1334153	COLNNNOT13	The library was constructed using RNA isolated from ascending colon tissue of a 28-year-old Caucasian male with moderate chronic ulcerative colitis.

Table 5

Program	Description	Reference	Parameter Threshold
ABI FACTURA	A program that removes vector sequences and masks ambiguous bases in nucleic acid sequences.	Perkin-Elmer Applied Biosystems, Foster City, CA.	
ABI/PARACEL FDF	A Fast Data Finder useful in comparing and annotating amino acid or nucleic acid sequences.	Perkin-Elmer Applied Biosystems, Foster City, CA; Paracel Inc., Pasadena, CA.	<i>ESTs</i> : Probability value= 1.0E-8 or less <i>Full Length sequences</i> : Probability value= 1.0E-10 or less
ABI AutoAssembler	A program that assembles nucleic acid sequences.	Perkin-Elmer Applied Biosystems, Foster City, CA.	
BLAST	A Basic Local Alignment Search Tool useful in sequence similarity search for amino acid and nucleic acid sequences. BLAST includes five functions: blastp, blastn, blastx, tblastn, and tblastx.	Altschul, S.F. et al. (1990) <i>J. Mol. Biol.</i> 215:403-410; Altschul, S.F. et al. (1997) <i>Nucleic Acids Res.</i> 25: 3389-3402.	<i>ESTs</i> : Probability value= 1.0E-8 or less <i>Assembled ESTs</i> : <i>fasta</i> Identity= 95% or greater and Match length=200 bases or greater; <i>fastx</i> E value= 1.0E-8 or less <i>Full Length sequences</i> : <i>fastx</i> score=100 or greater
FASTA	A Pearson and Lipman algorithm that searches for similarity between a query sequence and a group of sequences of the same type. FASTA comprises at least five functions: <i>fasta</i> , <i>tfasta</i> , <i>fastx</i> , <i>tfastx</i> , and <i>search</i> .	Pearson, W.R. and D.J. Lipman (1988) <i>Proc. Natl. Acad. Sci.</i> 85:2444-2448; Pearson, W.R. (1990) <i>Methods Enzymol.</i> 183: 63-98; and Smith, T.F. and M. S. Waterman (1981) <i>Adv. Appl. Math.</i> 2:482-489.	<i>ESTs</i> : <i>fasta</i> E value= 1.06E-6 <i>Assembled ESTs</i> : <i>fasta</i> Identity= 95% or greater and Match length=200 bases or greater; <i>fastx</i> E value= 1.0E-8 or less <i>Full Length sequences</i> : <i>fastx</i> score=100 or greater
BLIMPS	A BLocks IMProved Searcher that matches a sequence against those in BLOCKS and PRINTS databases to search for gene families, sequence homology, and structural fingerprint regions.	Henikoff, S and J.G. Henikoff, <i>Nucl. Acid Res.</i> , 19:6565-72, 1991. J.G. Henikoff and S. Henikoff (1996) <i>Methods Enzymol.</i> 266:88-105; and Altwood, T.K. et al. (1997) <i>J. Chem. Inf. Comput. Sci.</i> 37: 417-424.	Score=100 or greater; Ratio of Score/Strength = 0.75 or larger; and Probability value= 1.0E-3 or less
PFAM	A Hidden Markov Models-based application useful for protein family search.	Krogh, A. et al. (1994) <i>J. Mol. Biol.</i> , 235:1501-1531; Sonnhammer, E.L.L. et al. (1998) <i>Nucleic Acids Res.</i> 26:320-322.	Score=10-50 bits, depending on individual protein families

Table 5 (cont.)

Program	Description	Reference	Parameter Threshold
ProfileScan	An algorithm that searches for structural and sequence motifs in protein sequences that match sequence patterns defined in Prosite.	Gribskov, M. et al. (1988) CABIOS 4:61-66; Gribskov, et al. (1989) Methods Enzymol. 183:146-159; Bairoch, A. et al. (1997) Nucleic Acids Res. 25: 217-221.	Score= 4.0 or greater
Phred	A base-calling algorithm that examines automated sequencer traces with high sensitivity and probability.	Ewing, B. et al. (1998) Genome Res. 8:175-185; Ewing, B. and P. Green (1998) Genome Res. 8:186-194.	
Phrap	A Phils Revised Assembly Program including SWAT and CrossMatch, programs based on efficient implementation of the Smith-Waterman algorithm, useful in searching sequence homology and assembling DNA sequences.	Smith, T.F. and M. S. Waterman (1981) Adv. Appl. Math. 2:482-489; Smith, T.F. and M. S. Waterman (1981) J. Mol. Biol. 147:193-197; and Green, P., University of Washington, Seattle, WA.	Score= 120 or greater; Match length= 56 or greater
Consed	A graphical tool for viewing and editing Phrap assemblies	Gordon, D. et al. (1998) Genome Res. 8:195-202.	
SPScan	A weight matrix analysis program that scans protein sequences for the presence of secretory signal peptides.	Nielson, H. et al. (1997) Protein Engineering 10:1-6; Claverie, J.M. and S. Audic (1997) CABIOS 12: 431-439.	Score=5 or greater
Motif	A program that searches amino acid sequences for patterns that matched those defined in Prosite.	Bairoch et al. <u>supra</u> ; Wisconsin Package Program Manual, version 9, page M51-59, Genetics Computer Group, Madison, WI.	

What is claimed is:

1. A substantially purified polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:34, SEQ ID NO:35, SEQ ID NO:36, SEQ ID NO:37, SEQ ID NO:38, SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:41, SEQ ID NO:42, SEQ ID NO:43, SEQ ID NO:44, SEQ ID NO:45, SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:49, SEQ ID NO:50, SEQ ID NO:51, SEQ ID NO:52, SEQ ID NO:53, SEQ ID NO:54, SEQ ID NO:55, SEQ ID NO:56, SEQ ID NO:57, SEQ ID NO:58, SEQ ID NO:59, SEQ ID NO:60, SEQ ID NO:61, SEQ ID NO:62, SEQ ID NO:63, SEQ ID NO:64, SEQ ID NO:65, SEQ ID NO:66, SEQ ID NO:67, SEQ ID NO:68, SEQ ID NO:69, SEQ ID NO:70, SEQ ID NO:71, SEQ ID NO:72, SEQ ID NO:73, SEQ ID NO:74, SEQ ID NO:75, SEQ ID NO:76, SEQ ID NO:77, SEQ ID NO:78, SEQ ID NO:79, SEQ ID NO:80, SEQ ID NO:81, SEQ ID NO:82, SEQ ID NO:83, SEQ ID NO:84, SEQ ID NO:85, SEQ ID NO:86, SEQ ID NO:87, SEQ ID NO:88, SEQ ID NO:89, SEQ ID NO:90, SEQ ID NO:91, SEQ ID NO:92, SEQ ID NO:93, SEQ ID NO:94, SEQ ID NO:95, SEQ ID NO:96, SEQ ID NO:97, SEQ ID NO:98, SEQ ID NO:99, SEQ ID NO:100, SEQ ID NO:101, SEQ ID NO:102, SEQ ID NO:103, SEQ ID NO:104, SEQ ID NO:105, SEQ ID NO:106, SEQ ID NO:107, SEQ ID NO:108, SEQ ID NO:109, SEQ ID NO:110, SEQ ID NO:111, SEQ ID NO:112, SEQ ID NO:113, SEQ ID NO:114, SEQ ID NO:115, SEQ ID NO:116, SEQ ID NO:117, SEQ ID NO:118, SEQ ID NO:119, SEQ ID NO:120, SEQ ID NO:121, SEQ ID NO:122, SEQ ID NO:123, SEQ ID NO:124, SEQ ID NO:125, SEQ ID NO:126, SEQ ID NO:127, SEQ ID NO:128, SEQ ID NO:129, SEQ ID NO:130, SEQ ID NO:131, SEQ ID NO:132, SEQ ID NO:133, SEQ ID NO:134 (SEQ ID NO:1-134), and fragments thereof.

2. A substantially purified variant having at least 90% amino acid sequence identity to the amino acid sequence of claim 1.

3. An isolated and purified polynucleotide encoding the polypeptide of claim 1.
4. An isolated and purified polynucleotide variant having at least 90% polynucleotide sequence identity to the polynucleotide of claim 3.
5. An isolated and purified polynucleotide which hybridizes under stringent conditions to the polynucleotide of claim 3.
6. An isolated and purified polynucleotide having a sequence which is complementary to the polynucleotide of claim 3.
7. A method for detecting a polynucleotide, the method comprising the steps 10 of:
  - (a) hybridizing the polynucleotide of claim 6 to at least one nucleic acid in a sample, thereby forming a hybridization complex; and
  - (b) detecting the hybridization complex, wherein the presence of the hybridization complex correlates with the presence of the polynucleotide in the 15 sample.
8. The method of claim 7 further comprising amplifying the polynucleotide prior to hybridization.
9. An isolated and purified polynucleotide comprising a polynucleotide sequence selected from the group consisting of SEQ ID NO:135, SEQ ID NO:136, SEQ 20 ID NO:137, SEQ ID NO:138, SEQ ID NO:139, SEQ ID NO:140, SEQ ID NO:141, SEQ ID NO:142, SEQ ID NO:143, SEQ ID NO:144, SEQ ID NO:145, SEQ ID NO:146, SEQ ID NO:147, SEQ ID NO:148, SEQ ID NO:149, SEQ ID NO:150, SEQ ID NO:151, SEQ ID NO:152, SEQ ID NO:153, SEQ ID NO:154, SEQ ID NO:155, SEQ ID NO:156, SEQ ID NO:157, SEQ ID NO:158, SEQ ID NO:159, SEQ ID NO:160, SEQ ID NO:161, SEQ 25 ID NO:162, SEQ ID NO:163, SEQ ID NO:164, SEQ ID NO:165, SEQ ID NO:166, SEQ ID NO:167, SEQ ID NO:168, SEQ ID NO:169, SEQ ID NO:170, SEQ ID NO:171, SEQ ID NO:172, SEQ ID NO:173, SEQ ID NO:174, SEQ ID NO:175, SEQ ID NO:176, SEQ ID NO:177, SEQ ID NO:178, SEQ ID NO:179, SEQ ID NO:180, SEQ ID NO:181, SEQ ID NO:182, SEQ ID NO:183, SEQ ID NO:184, SEQ ID NO:185, SEQ ID NO:186, SEQ 30 ID NO:187, SEQ ID NO:188, SEQ ID NO:189, SEQ ID NO:190, SEQ ID NO:191, SEQ ID NO:192, SEQ ID NO:193, SEQ ID NO:194, SEQ ID NO:195, SEQ ID NO:196, SEQ ID NO:197, SEQ ID NO:198, SEQ ID NO:199, SEQ ID NO:200, SEQ ID NO:201, SEQ

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15 10. An isolated and purified polynucleotide variant having at least 90% polynucleotide sequence identity to the polynucleotide of claim 9.

11. An isolated and purified polynucleotide having a sequence which is complementary to the polynucleotide of claim 9.

12. An expression vector comprising at least a fragment of the polynucleotide 20 of claim 3.

13. A host cell comprising the expression vector of claim 12.

14. A method for producing a polypeptide, the method comprising the steps of:

a) culturing the host cell of claim 13 under conditions suitable for the expression of the polypeptide; and

25 b) recovering the polypeptide from the host cell culture.

15. A pharmaceutical composition comprising the polypeptide of claim 1 in conjunction with a suitable pharmaceutical carrier.

16. A purified antibody which specifically binds to the polypeptide of claim 1.

17. A purified agonist of the polypeptide of claim 1.

30 18. A purified antagonist of the polypeptide of claim 1.

19. A method for treating or preventing a disorder associated with decreased expression or activity of HSPP, the method comprising administering to a subject in need of such treatment an effective amount of the pharmaceutical composition of claim 15.

20. A method for treating or preventing a disorder associated with increased expression or activity of HSPP, the method comprising administering to a subject in need of such treatment an effective amount of the antagonist of claim 18.

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